INHIBITION OF SACRAL PARASYMPATHETIC PREGANGLIONIC NEURONS BY GABA, GLYCINE, 5-HYDROXYTRYPTAMINE AND NOREPINEPHRINE

by

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ABSTRACT

Sacral parasympathetic preganglionic discharges recorded from the pelvic nerve were evoked by stimulation of sacral afferent fibers, descending pathways in the dorsolateral spinal cord, or medullary vesico-constrictor centers. Evoked activity was summed and integrated with a signal averaging computer in 67 spinal or decerebrate cats. Coadministration of picrotoxin (2 mg/kg) and strychnine (0.2 mg/kg), but neither alone, increased evoked activity by 4 to 10 times. In addition, after treatment with both picrotoxin and strychnine the increases in bladder pressures evoked by stimulus trains were enhanced by 2-12 times. Bicuculline (0.5 mg/kg) could be substituted for picrotoxin. Similar increases in evoked pelvic nerve activity were induced by strychnine (0.1 mg/kg) in cats depleted of GABA stores with semicarbazide (200 mg/kg). D,L-5-HTP (50 mg/kg) or L-dopa (35 mg/kg) depressed evoked activity in both normal and convulsant-treated cats. Depression by 5-HTP or L-dopa was augmented by chlorimipramine or protriptyline, respectively. These results indicate that sacral parasympathetic preganglionic neurons are under strong, local tonic inhibition mediated by both GABA and glycine. The 5-HT and NE bulbospinal neurons that terminate in the intermediolateral portion of the sacral spinal cord also appear to exert an inhibitory influence. The results are

compatible with clinical experience in patients with loss of bladder function due to lesions of the spinal cord.

INTRODUCTION

The existence of bulbospinal neuron systems containing norepinephrine (NE) and 5-hydroxytryptamine (5-HT) was demonstrated by Dahlström and Fuxe with a specific histochemical fluorescence technic (12). Sympathetic preganglionic neurons in the thoracic intermediolateral portion of the spinal cord were found to receive a rich supply of both NE and 5-HT terminals whereas the sacral parasympathetic preganglionic neurons in the sacral intermediolateral columns receives a smaller supply of monoaminergic terminals which are predominantly 5-HT. Although the transmitter stores of these monoaminergic neurons were increased or depleted, as determined by their fluorescence intensity, by drugs affecting either monoamine metabolism or reuptake mechanisms, their effects on sympathetic or parasympathetic preganglionic neurons were not determined (14).

As the respective roles of bulbospinal NE and 5-HT neurons at the thoracic sympathetic preganglionic neurons have since been tentatively established (38), the purpose of this investigation was to determine the effects of NE and 5-HT neurons on sacral parasympathetic preganglionic activity. In addition to obtaining evidence for the transmitter roles of NE and 5-HT on sacral parasympathetic preganglionic neurons, evidence for the important roles of the putative inhibitory

transmitters, gamma-aminobutyric acid (GABA) and glycine, was also found. Characterization of the sacral autonomic outflow and of its excitatory inputs has also been extended.

METHODS

Surgical procedures

Results were obtained from 67 unanesthetized, spinal or decerebrate cats of either sex weighing 2.3-5.0 kg. Under a brief ether anesthesia, the common carotid arteries were ligated and one was cannulated for recording blood pressure. Anesthesia was discontinued when the cats were made spinal or decerebrate, and skeletal muscle paralysis was maintained with gallamine triethiodide (Flaxedil). The vertebral arteries were clamped in spinal preparations. The animals were ventilated through a tracheal cannula with positive pressure respiration adjusted to maintain end tidal CO_2 concentration between 3.5-4.5% (Spinco Medical Gas Analyzer). Drug solutions and supporting fluids were administered through a catheter placed in a cephalic vein. Deep body temperature, monitored by a rectal thermistor, was automatically maintained at 37-38^{o} by an abdominal heating plate. Mean blood pressures in spinal and decerebrate preparations were generally about 70 and 120 mm Hg, respectively. Saline (0.9%), 5% dextrose or 10% dextran-40 were infused as necessary to maintain blood pressures above 70 mm Hg.

In experiments not involving medullary stimulation, cats were made spinal by a C_1 transection. Investigation of the medullary centers was

conducted in animals decerebrated by a brain stem lesion made rostral to the tentorium either electrolytically or surgically. Spinal parasympathetic reflexes were evoked by stimulating isolated, sacral somatic nerves or sacral dorsal roots exposed by a dorsolateral laminectomy. The descending spinal pathway in the dorsolateral funiculus was approached through a T_7-T_9 dorsolateral laminectomy. Access to sites of medullary stimulation was obtained by partial removal of occipital bone and, occasionally, by removal of the caudal cerebellum.

The pelvic nerve containing parasympathetic preganglionic axons was isolated by a dorsal approach through the greater sciatic notch; identification of the pelvic nerve was verified by its position and by its entrance into the extensive pelvic plexus. In order to free as much nerve as possible for recording, sections of the lateral sacral vertebrae and the overlying sacral somatic nerves were removed. After the pelvic nerve was exposed and isolated, the surrounding connective tissue was carefully removed by microdissection and a polyethylene retainer was placed under the recording site to restrict movement of the rectum. The nerve was severed near its entry into the pelvic plexus, and the central portion was mounted on bipolar electrodes for recording; the contralateral pelvic nerve remained intact.

In several experiments, a cannula was inserted into the bladder through a suprapubic incision of the urethra and secured with a ligature. Care was taken to avoid interference with the nerve supply to the

bladder. The cannula was attached through a stopcock to a vertical glass rod calibrated in cm from which bladder pressure was monitored; the stopcock allowed saline to be introduced into or removed from the bladder as necessary.

Removal of muscle tissue was expedited by cautery, and small blood vessels were sealed with a microcoagulator. A binocular dissecting microscope (3-25X) was used for delicate procedures. Exposed tissues at stimulating and recording sites were covered with a pool of light mineral oil.

Stimulating and recording technics

Parasympathetic preganglionic activity recorded from the pelvic nerve with bipolar, silver wire electrodes was evoked by stimulation at several sites (Fig. 1). Responses were evoked by supramaximal rectangular pulses (0.2 msec) delivered by an isolated stimulator (Devices, Mk IV) at a frequency of 12/min; at this frequency the responses were quite stable for several hours. In reflex experiments sacral somatic nerves or sacral dorsal roots were stimulated through bipolar, silver wire electrodes. Descending spinal pathways and medullary centers were stimulated through bipolar tungsten microelectrodes (tip exposure 25 μm, 3-6 MΩ, tip separation 1 mm). Microelectrodes were positioned with a micromanipulator under microscopic control. Locations of the respective spinal pathways and medullary

centers were established in initial experiments so that proper electrode placement was readily accomplished.

Evoked pelvic nerve discharges were amplified by a Tektronix 2A61 preamplifier set at a frequency response of 0.6-600 Hz and were displayed on a dual-beam oscilloscope. Since recorded activity in untreated animals often had a low signal/noise ratio, a summed analogue display of 32 consecutive evoked discharges was obtained with a signal averaging computer (Nicolet 1072). Integration of summed responses by the computer provided on-line analysis of all data. The integrated areas were normalized by conversion to percentages of mean control values. The analogue display and several individual responses were photographed from the oscilloscope on 35 mm orthographic film. A crystal clock timing device (Digitimer, Devices) controlled triggering of the stimulating and recording equipment.

Experimental procedures

Control recordings from the pelvic nerve usually began four to six hours following spinal cord transection or decerebration. The length of the recording period was determined by several factors, including the condition of the animal (evaluated by carotid blood pressure) and the particular experimental procedure. This period was frequently greater than seven hours and in several cases exceeded twenty hours. Control measurements were collected at 5 minute intervals, and drugs were

tested only after at least one hour of stable controls were recorded. Following drug administration, measurements were made at 5 minute intervals until the drug effect stabilized; during stable periods data were collected at 10 or 20 minute intervals. In several experiments the bladder pressure response to a brief stimulus train (20/sec for 2 sec) was measured before and after the administration of convulsants.

Phenoxybenzamine (0.3-1.0 mg/kg) prevented the large increases in blood pressure in animals treated with L-dopa or convulsants. Phenoxybenzamine and gallamine had no effect on sacral parasympathetic preganglionic outflow.

Each type of experiment reported was performed in at least three cats unless otherwise noted. Although quantitatively the response to drugs differed slightly among experiments, the qualitative changes were very consistent.

Drug solutions

Drugs were administered by slow intravenous injection except for parachlorophenylalanine (PCPA) which was suspended in 0.9% NaCl solution with a small amount of Tween 80 and injected intraperitoneally. Phenoxybenzamine was dissolved in propylene glycol and diluted to 1 mg/ml with 0.9% NaCl solution. Strychnine and picrotoxin were prepared as stock solutions of 1 mg/ml and 3 mg/ml, respectively, in 0.9% NaCl solution. Bicuculline was dissolved in 0.1 N HCl and

diluted with 0.9% NaCl solution. Semicarbazide was administered as a 50 mg/ml solution. All other drugs were either dissolved in 0.9% NaCl solution (10 mg/kg) or were available as commercial preparations for intravenous injection.

RESULTS

Sacral parasympathetic preganglionic neurons

The axons of sacral parasympathetic preganglionic neurons emerge from cell bodies in the sacral parasympathetic nucleus of the spinal cord and are carried by the pelvic nerves to their terminations in parasympathetic ganglia in the bladder, urethra, descending colon, rectum, and sexual organs (20,32,33,34,35,36). The majority of these axons innervate the detrusor muscle of the bladder, where their activation produces contraction of the detrusor muscle and elevation of bladder pressure. The sacral autonomic neurons are innervated by peripheral afferent fibers originating primarily from receptors in the various organs of the pelvic viscera and by bulbospinal pathways. The locations of the pertinent central and peripheral pathways and their influences on the output of sacral parasympathetic preganglionic motor neurons as recorded from the pelvic nerve were investigated initially to determine their physiological characteristics.

Bulbospinal pathways. Two distinct pairs of medullary micturition centers, the bulbar vesico-constrictor and the vesico-relaxer centers, have been described by Kuru (30,31). These centers are located bilaterally in the medulla oblongata, and their descending axons provide

efferent connections to the sacral autonomic nucleus. Stimulation of the vesico-constrictor centers in the lateral reticular nucleus with bipolar microelectrodes evoked both pelvic nerve discharges and bladder contraction. Maximum responses were evoked by stimulation in a well-defined area that corresponds to the location of the vesico-constrictor center described by Kuru.

Although vesico-constrictor fibers descend to the sacral parasympathetic neurons by rather diffuse pathways in the dorsolateral funiculus of the spinal cord, the pathways are most discretely organized near the eighth thoracic segment (T_8). Stimulation in this region with bipolar microelectrodes about 1 mm lateral to the dorsal root entry zone and at a depth of about 1 mm was most effective in evoking responses from the pelvic nerve. Bladder contraction and pelvic nerve activity could be evoked either contralaterally or ipsilaterally from the medullary centers or from the spinal cord pathways since the descending fibers from the vesico-constrictor centers partially decussate in the lumbar spinal cord.

The lack of appreciable spontaneous pelvic nerve activity and the normally low bladder pressures rendered precise location of the bulbar vesico-relaxer centers more difficult. Nevertheless, artificially increased bladder pressures were generally reduced 2-5 cm H_2O by microelectrode stimulation of the dorsomedial reticular formation with brief trains of impulses (20/sec for 2 sec). This area corresponds to the location of the vesico-relaxer center described by Kuru.

Spinal reflex pathways. Peripheral sacral autonomic and somatic afferent fibers provide important sources of inhibitory and excitatory influence on sacral autonomic neurons that innervate the bladder (1,2, 3,16). Many of the inhibitory afferent fibers originate from receptors in the anus, sexual organs and bladder, whereas the excitatory fibers are primarily derived from the urethra and bladder. Although spinal parasympathetic reflexes could be evoked by stimulation of either sacral dorsal roots or sacral somatic nerves, the sacral dorsal roots provided a much better source of excitatory drive than did the sacral somatic nerves, presumably because the dorsal roots contained excitatory parasympathetic afferents from the bladder and urethra.

Characteristics of pelvic nerve discharges. Stimulation of medullary vesico-constrictor centers, descending spinal pathways, or spinal reflex pathways produced similar evoked discharges recorded from the pelvic nerve. Evoked discharges, summed by a signal averaging computer, appeared as long, diffuse waves of 100-150 msec duration (Fig. 2A,B,C). Responses evoked from the medullary centers or descending spinal pathways (T_8) had latencies of 60 and 40 msec, respectively. The latency of the spinal reflexes was about 40 msec. The conduction velocity of bulbosacral pathways estimated from both the vesico-constrictor areas in the medulla and the excitatory pathways in the spinal cord at T_8 was 4.5-5.5 m/sec.

The discharges evoked from stimulation at any of the three sites (medulla, spinal cord, or dorsal roots) and recorded from the pelvic nerve were usually very small, and were often barely detectable without the aid of the averaging computer. Therefore, the possibility that parasympathetic preganglionic neurons are under some type of tonic inhibition was considered. The influence of a peripheral inhibitory tone was ruled out after a complete lumbar and sacral bilateral dorsal rhizotomy failed to increase the evoked activity. However, stimulation of the S_2 ventral root, which contains the majority of efferent axons in the pelvic nerve, produced a very large discharge. This finding confirmed the patency of the preganglionic axons and indicated that the proportion of available preganglionic neurons discharged by reflex or central activation was normally quite small. Since the evoked responses remained small in animals spinalized at the cervical or lower thoracic spinal cord and were not influenced by dorsal rhizotomy, it was tentatively concluded that the sacral parasympathetic preganglionic neurons are under the influence of a strong, local tonic inhibition.

Influence of gamma-aminobutyric acid (GABA) and glycine on sacral autonomic neurons

Since the preliminary investigations suggested the presence of local tonic inhibition of sacral autonomic neurons, the effects of several

antagonists of putative inhibitory neurotransmitters were tested in an effort to reveal the nature of this inhibition.

Picrotoxin and strychnine. Picrotoxin and strychnine are known to block the effects of the inhibitory amino acids GABA and glycine, respectively, at various invertebrate and mammalian synapses (28). Repeated administration of picrotoxin (0.5-2.0 mg/kg) only slightly increased the size of pelvic nerve discharges evoked by stimulation of medullary centers, intraspinal pathways, or sacral dorsal roots. Spontaneous pelvic nerve activity was also increased only slightly. However, the additional administration of strychnine sulfate (0.05-0.20 mg/kg) rapidly and markedly increased the size of evoked discharges (Figs. 3A and 4) and the frequency of spontaneous activity. If the order of drug administration was reversed, strychnine alone elicited only small increases in evoked discharges or spontaneous activity but subsequent administration of picrotoxin produced large increases in evoked discharges (Figs. 3B and 5) and spontaneous activity.

In general, the effects of small incremental doses of the second convulsant were additive to a point of maximum effect beyond which no further increases in evoked responses could be produced by additional drug. This dose-response relationship was apparent over the range of doses listed above. In more than 30 experiments administration of both picrotoxin and strychnine, but neither alone, produced a mean increase in evoked pelvic nerve activity of 400% of control values; in several

preparations the increase exceeded 1000%. Administration of the second convulsant rapidly (1-5 min) increased the size of evoked pelvic nerve discharges. In most experiments the increase in evoked activity either remained at a peak level or stabilized at a lower value for a period of one to eight hours. Often, second doses of both picrotoxin and strychnine produced further increases in evoked responses, especially after the initial maximal increase had declined.

No changes in latencies were observed, but the duration of responses frequently increased by 50-100 msec after the convulsants. No consistent differences in response to the coadministration of convulsants were observed among the medullary, intraspinal or spinal reflex pathway experiments or between the order of convulsant administration. These results indicate that local tonic inhibition of sacral parasympathetic preganglionic neurons is mediated by both GABA and glycine, and that this inhibition can only be removed by simultaneous blockade of both inhibitory amino acids.

Bicuculline and strychnine. Since bicuculline has been demonstrated to be about four times more potent than picrotoxin as a GABA antagonist (28), its ability to block the inhibition of sacral autonomic neurons alone and in conjunction with strychnine was investigated. Large increases in evoked pelvic nerve discharges and spontaneous activity were obtained only after both bicuculline (0.5 mg/kg) and strychnine were administered (Figs. 6A and 7). Bicuculline alone was

as ineffective as picrotoxin alone. The increases in preganglionic discharges evoked by the different pathways were similar and were not influenced by the order of drug administration. In general, no differences were observed between the efficacies of bicuculline and picrotoxin.

Semicarbazide and strychnine. Inhibition of GABA synthesis by semicarbazide produces an almost total depletion of central GABA stores within four hours after administration (4). Therefore, the effect of semicarbazide administration should be equivalent to the effect of picrotoxin or bicuculline administration. Additionally, the subsequent injection of strychnine would effect the same response as the convulsant combination. In two experiments utilizing bulbosacral and spinal reflex pathways, an increase (50%) in evoked pelvic nerve activity slowly developed following semicarbazide (200 mg/kg) administration (Fig. 6B), and resembled the small increases observed after picrotoxin. Four hours after semicarbazide treatment, administration of strychnine (0.1 mg/kg) produced large increases (350-600%) in evoked parasympathetic discharges (Figs. 6B and 8); these effects of strychnine could not be differentiated from those obtained following the picrotoxin-strychnine combination. These results provided further evidence for tonic inhibition of the sacral parasympathetic preganglionic neurons by inhibitory systems utilizing both GABA and glycine as transmitters.

Evoked bladder pressure. In nine experiments changes in evoked bladder pressure, measured through a urethral cannula, were compared with changes in evoked pelvic nerve discharges. Resting bladder pressure was maintained at about 10 cm H_2O by regulating the amount of fluid in the bladder. The usual response of the bladder to a train of stimuli in untreated animals was an increase in pressure of 3-5 cm H_2O. However, after treatment with both picrotoxin and strychnine, the increments in evoked bladder pressure increased 2-12 times; spontaneous bladder contractions also increased in magnitude and frequency. Thus, the activity recorded from the pelvic nerve, even though comprised of fibers innervating several different organs, was well correlated with the excitatory motor response of the bladder. Furthermore, it should be noted that the large increases in bladder pressure after the convulsant drug combination were achieved despite the loss of innervation by the ipsilateral pelvic nerve from which preganglionic activity was recorded.

Effect of monoamines on sacral autonomic neurons

Histochemical fluorescence studies have demonstrated that parasympathetic preganglionic neurons located in the sacral intermediolateral columns appear to receive terminals containing norepinephrine (NE) and, predominately, 5-hydroxytryptamine (5-HT) (13,14,26). Although these monoaminergic fibers originate in the medulla oblongata and terminate throughout the spinal gray matter, they appear to be

highly concentrated in the sympathetic and parasympathetic autonomic nuclei of the thoracic and sacral spinal cord, respectively. A number of experiments were conducted with monoamine precursors and drugs affecting central monoaminergic transmission in order to determine the influence of monoamines on sacral parasympathetic preganglionic activity.

5-Hydroxytryptophan (5-HTP). The influence of 5-HT terminals on sacral autonomic neurons was initially investigated by administration of its precursor, 5-HTP. Following intravenous injection, 5-HTP rapidly passes into the central nervous system and is taken up by serotonergic neurons where it is converted to 5-HT by aromatic L-amino acid decarboxylase (22). Since the stores of 5-HT are rapidly increased in this manner (12), some spill-over into the synapse occurs (9) allowing 5-HT to exert its normal effect postsynaptically.

Administration of 5-HTP (25-50 mg/kg) decreased the pelvic nerve discharges evoked from bulbosacral or spinal reflex pathways. The active isomer, L-5-HTP, was more effective than D,L-5-HTP. Treatment with 12.5 mg/kg of L-5-HTP produced a small but significant decrease in evoked pelvic nerve activity, whereas the same dose of D,L-5-HTP was without effect. In general, 50 mg/kg of D,L-5-HTP gradually depressed the evoked pelvic nerve response to about 50% of control values over a period of 25-50 min (Fig. 9A). The depression produced by this dose often showed little recovery for 3-5 hours, but the effects of smaller doses (25-35 mg/kg) usually waned after two hours.

The effect of 5-HTP on large, evoked pelvic nerve discharges in convulsant-treated animals was also investigated in bulbosacral and spinal reflex pathway experiments. After the picrotoxin-strychnine-induced increase in evoked pelvic nerve activity had stabilized, administration of 5-HTP (50 mg/kg) produced a gradual decrease in evoked responses; this effect of 5-HTP was similar in all respects to the effect of 5-HTP in untreated animals (Fig. 10A).

Monoamine oxidase inhibition. Inhibition of monoamine oxidase allows intraneuronal 5-HT to increase markedly in serotonergic neurons, especially after administration of precursors (9,12). Treatment with the monoamine oxidase inhibitor, pargyline (30 mg/kg), quickly (10-20 min) decreased evoked pelvic nerve responses to 50-70% of control values where they remained with little or no recovery for at least 3 hours. After the depressant effects of pargyline had stabilized, the administration of L-tryptophan (150 mg/kg) or 5-HTP (5 mg/kg) depressed responses evoked from bulbosacral or spinal reflex pathways by an additional 40-50% (Fig. 9B). In untreated animals 5 mg/kg of 5-HTP produced no significant changes in the evoked pelvic nerve discharges; thus, the inhibition of monoamine oxidase increased the potency of 5-HTP on sacral parasympathetic neurons as much as 10 times.

Chlorimipramine. The presynaptic reuptake of 5-HT is selectively inhibited by the tricyclic antidepressant, chlorimipramine (6,7,11,23, 37). The depression of activity evoked from bulbosacral or spinal reflex

pathways in untreated or convulsant-treated animals by 5-HTP (25-50 mg/kg) was enhanced by subsequent administration of chlorimipramine (5 mg/kg) (Fig. 10A). Chlorimipramine (5 mg/kg) alone produced a gradual decrease in pelvic nerve activity evoked from bulbosacral or spinal reflex pathways (50% of control) in both untreated and convulsant-treated animals. The addition of 5-HTP (35 mg/kg) further depressed (30% of control values) the discharges evoked from bulbosacral or spinal reflex pathways (Fig. 10B). Potentiation of the inhibitory effects of 5-HTP on sacral autonomic neurons by chlorimipramine can be explained by inhibition of the 5-HT reuptake mechanism.

Clonidine. Inhibition of sympathetic preganglionic neurons via stimulation of 5-HT receptors may explain the central anti-hypertensive action of clonidine (38). The clonidine-induced depression of sympathetic neurons in untreated and reserpinized cats was reversed by tolazoline, which has been demonstrated to antagonize 5-HT-mediated inhibition of sympathetic preganglionic neurons. Clonidine (10 μg/kg) decreased pelvic nerve responses evoked from bulbosacral or spinal reflex pathways to about 50% of control values, and this depression of evoked discharges persisted. Tolazoline (2 mg/kg) rapidly reversed the depression back to or near control values (Fig. 11). These data support the evidence obtained from sympathetic neurons that clonidine may produce its action through stimulation of central serotonergic receptors.

L-dopa. The influence of NE fibers terminating on sacral parasympathetic preganglionic neurons was also studied by testing the effect of L-dopa on pelvic nerve discharges evoked by bulbospinal or spinal reflex pathways. Intraneuronal stores of NE are increased and spill-over into the synaptic cleft occurs after the administration of L-dopa (9,12). L-dopa readily passes into the central nervous system where it is taken up by adrenergic terminals and converted into NE.

Administration of L-dopa (35 mg/kg) gradually decreased pelvic nerve discharges evoked from spinal reflex pathways to about 50% of control values. This dose of L-dopa produced a maximum depression of evoked activity in 20-30 min, and in most experiments the response returned almost to control values within 2 to 3 hours (Fig. 12). A lower dose of L-dopa (25 mg/kg) produced depression of evoked activity that was smaller in magnitude and duration. Administration of D-dopa (35 mg/kg) failed to change evoked pelvic nerve discharges; L-dopa is the active isomer.

The effects of L-dopa on evoked pelvic nerve responses in convulsant-treated animals were qualitatively the same as those obtained in untreated animals. After the pelvic nerve activity evoked from bulbosacral or spinal reflex pathways was increased by coadministration of picrotoxin and strychnine, L-dopa (35 mg/kg) steadily decreased the evoked responses. The peak depression of evoked activity was obtained in 20-30 min and then gradually reversed. No differences in the effect

of L-dopa were noted between untreated or convulsant-treated preparations.

PCPA and tolazoline. Although NE appears to have an excitatory action on thoracic sympathetic preganglionic neurons, an initial depression of sympathetic neurons produced by L-dopa administration is attributed to L-dopa-induced displacement of 5-HT from serotonergic terminals (38). Therefore, several experiments were conducted to determine whether the inhibition of sacral parasympathetic preganglionic neurons by L-dopa was a direct (NE) or an indirect (5-HT release) effect.

Tolazoline (1.5 mg/kg) quickly reversed the depression of thoracic sympathetic preganglionic neurons produced by L-dopa administration, and pretreatment with tolazoline (5 mg/kg) completely prevents the L-dopa-induced depression of evoked sympathetic preganglionic responses (38). Tolazoline is thought to antagonize the 5-HT released by L-dopa administration.

Administration of tolazoline (5 mg/kg) either had no effect or slightly decreased pelvic nerve activity evoked from bulbospinal pathways in convulsant-treated animals, but subsequent administration of L-dopa (35 mg/kg) gradually depressed the evoked pelvic nerve activity (Fig. 13A). The times to peak effect and the reversal towards control values were almost identical to that observed in animals that were not pretreated with tolazoline. These results indicate that L-dopa was producing its effects directly via NE.

In two cats the tryptophan hydroxylase inhibitor, PCPA (100 mg/kg), was administered intraperitoneally for three consecutive days, and the experiments were conducted on the fourth day. This treatment schedule has been demonstrated to decrease greatly spinal cord stores of 5-HT without affecting catecholamine levels (27). Since the amount of pelvic nerve activity evoked from bulbosacral pathways was as small in 5-HT-depleted cats as in untreated animals, the evoked responses were increased with picrotoxin and strychnine treatment. The administration of L-dopa (35 mg/kg) produced a gradual depression of evoked pelvic nerve activity in 5-HT-depleted animals that was similar to that obtained in untreated animals (Fig. 13B). These results suggest that depression of pelvic nerve activity following administration of L-dopa was mediated directly by NE.

Protriptyline. Reuptake of NE by adrenergic terminals is selectively blocked by the tricyclic antidepressant, protriptyline (8,10,23). The depression of discharges evoked from spinal reflex pathways in untreated or convulsant-treated animals produced by relatively small doses of L-dopa (25 mg/kg) was potentiated by administration of protriptyline (5 mg/kg) (Fig. 14A). Administration of protriptyline (5 mg/kg) to untreated or convulsant-treated animals produced a 20-40% decrease in pelvic nerve activity evoked from spinal reflex pathways, and subsequent administration of L-dopa (25-30 mg/kg) further depressed the evoked responses which usually slowly reversed to or near pretreatment

values (Fig. 14B). These results were attributed to the persistence of extraneuronal NE following inhibition of NE reuptake with protriptyline.

Reserpine. Inhibition of the uptake mechanism for monoamines into storage granules by reserpine produces a biphasic effect: initially, monoamines are released into the synapse and mimic stimulation of monoaminergic neurons (5,24),and secondly, after depletion of monoamine stores, the monoaminergic influence disappears (26). Since both NE and 5-HT appeared to mediate direct inhibitory effects on sacral parasympathetic preganglionic neurons, the effect of their release by reserpine was tested to obtain further support for this hypothesis.

Administration of reserpine (5 mg/kg) rapidly depressed the large, evoked pelvic nerve discharges produced by pretreatment with picrotoxin and strychnine (Fig. 15). Furthermore, administration of additional picrotoxin and strychnine during the depressant effect of reserpine failed to reverse the depression. These effects were seen regardless of whether pelvic nerve responses were evoked from bulbosacral or spinal reflex pathways. These results indicate that the reserpine-induced depression was not due to a loss of convulsant effects but rather was due to release of NE and 5-HT.

DISCUSSION

The results of the present investigation confirm and extend present understanding of the central and peripheral control of sacral parasympathetic preganglionic neurons, in particular those that innervate the urinary bladder. Additional characterization of central and peripheral excitatory pathways has been obtained, and considerable evidence for the functions of several inhibitory systems and their neurotransmitters has been gained.

Langley and Anderson (32,33,34,35), and later Elliot (20), provide the first detailed descriptions concerning the anatomy and function of the sacral parasympathetic innervation of the pelvic viscera in cats. Parasympathetic preganglionic motor fibers are distributed by the pelvic nerves to the descending colon, rectum, bladder, urethra and sexual organs, where they synapse on short postganglionic neurons. The sacral parasympathetic outflow is organized primarily for discrete and localized control of vegetative functions. Cell bodies of parasympathetic preganglionic axons are located in the intermediolateral columns of the second and third sacral spinal segments; this position corresponds to the location of the sympathetic nucleus in the thoracic and lumbar cord (40). The location of parasympathetic preganglionic neurons has been recently verified by observing retrograde changes following pelvic

nerve section (39) and by recording antidromic potentials elicited by stimulation of the pelvic nerves or the second or third sacral ventral roots with intracellular and extracellular microelectrodes (17). Although the pelvic nerve contains the parasympathetic motor supply to several different organs, an excellent correlation between evoked pelvic nerve activity and evoked increases in bladder pressure was found in the present experiments.

Sacral parasympathetic neurons are innervated by a variety of excitatory and inhibitory axon terminals from both peripheral receptors and higher brain centers. Spinal reflex pathways exert a modest influence on these neurons (1,2,3,16,18) but are normally inadequate to produce micturition; neural mechanisms for micturition remain inoperable for weeks following acute spinal transection. Normal micturition is largely controlled by supraspinal centers and is dependent upon intact sensory and motor pathways in the spinal cord.

The presence of micturition centers in the brain stem has been alluded to by many investigators, but Kuru (30,31) actually localized and characterized two micturition centers in the medulla of the cat, the bulbar vesico-constrictor and vesico-relaxer centers. Axons from the vesico-constrictor center, located in the lateral reticular nuclei, descend in the lateral reticulospinal tract. Fibers from the vesico-relaxer center, located in the dorsomedial reticular formation, descend in the ventral reticulospinal tract. Both centers and their respective descending pathways are located bilaterally in the medulla and spinal

cord, but their axons partially decussate at the lumbar level before terminating in the intermediolateral columns of the sacral spinal cord. The existence and locations of these areas were verified in the present investigation. Stimulation of a vesico-constrictor center or its descending pathway in the dorsolateral funiculus at T_8 produced small increases in bladder pressure whereas stimulation of a vesico-relaxer center often caused small decreases in bladder pressure. In the non-convulsant-treated animal, however, pelvic nerve discharges evoked from either the vesico-constrictor centers or their descending spinal pathways were quite small. Several supramedullary areas in the pons, mesencephalon, hypothalamus, septum pellucidum, amygdala and cerebral cortex are also concerned with micturition (29), but their influence appears to be less important than that arising from the medullary centers. Therefore, micturition appears to be a highly integrated response controlled by a number of higher brain centers that are influenced by appropriate sensory activation.

Dahlström and Fuxe (13,14) describe NE and 5-HT bulbospinal neurons systems in the cat that project to the sacral autonomic nucleus where they appear to make synaptic contact with parasympathetic preganglionic neurons; the 5-HT system is predominant. Although these pathways originate from cell bodies in the medulla and terminate in the sacral autonomic nucleus, their possible functions have remained unknown. The cell bodies of the descending NE neuron system are

localized mainly in the ventrolateral reticular formation of the medulla oblongata. Those of the 5-HT neuron system are found in the three raphe nuclei of the medulla and in the part of the reticular formation immediately surrounding the pyramidal tract at this level. Both NE and 5-HT pathways descend to the intermediolateral columns in the dorsolateral funiculus of the spinal cord.

Kuru's vesico-relaxer center is readily distinguished from either of the monoaminergic neuron systems both by its medullary location and by the position of its descending spinal pathways. However, a distinction between the vesico-constrictor center and its descending spinal pathways and the NE neuron system is not apparent anatomically. These two neuron systems were determined to be separate by the nature of their influence on sacral parasympathetic preganglionic neurons; the vesico-constrictor centers mediate excitation of sacral autonomic neurons whereas the NE terminals appear to have an inhibitory action on these cells.

The results of the present investigation strongly suggest inhibitory roles for both NE and 5-HT bulbospinal neurons on sacral parasympathetic preganglionic neurons. The consistent depression of evoked pelvic nerve activity following administration of small doses of either L-dopa or 5-HTP can be explained by their ability to increase terminal monoamine stores that ultimately spill into the synaptic cleft to produce their characteristic effects. That the depression of evoked activity is

produced by NE or 5-HT rather than by their precursors is indicated by the relatively slow onset and by the time course to peak depression. In addition, inhibition of monoamine oxidase permits substantial depression of evoked activity by doses of 5-HTP and L-tryptophan that are normally ineffective. In contrast to sympathetic preganglionic neurons where L-dopa produces an initial depression due to 5-HT displacement (38), inhibition of sacral autonomic neurons by L-dopa appears to be mediated directly by NE; neither postsynaptic block of 5-HT receptors by tolazoline or depletion of 5-HT by PCPA alter the nature of L-dopa-induced depression of sacral autonomic neurons. Tricyclic antidepressants that selectively inhibit the reuptake of NE or 5-HT not only decrease evoked pelvic nerve activity alone but also tend to potentiate the depression produced by administration of the respective precursors. Finally, reserpine, by causing an initial release of monoamines (5), strongly depressed evoked pelvic nerve activity. The apparent inhibitory transmitter role of NE on sacral parasympathetic preganglionic neurons differs from that observed on sympathetic preganglionic neurons where NE is excitatory. On the other hand, 5-HT appears to mediate inhibition at both sites.

Although the monoamines are inhibitory to sacral parasympathetic preganglionic neurons, the major inhibitory influence appears to be mediated by both GABA and glycine. Only the concomitant administration of the glycine antagonist, strychnine, and an antagonist of GABA, either

bicuculline or picrotoxin, is sufficient to produce disinhibition of the sacral autonomic neurons so that excitatory drive from either the higher centers or the periphery becomes many times more effective. The evidence for such glycine-GABA-mediated inhibition is further supported by experiments in which GABA synthesis was inhibited by semicarbazide. After depletion of GABA, administration of strychnine alone is adequate to disinhibit the parasympathetic preganglionic neurons. The strong inhibition of parasympathetic preganglionic neurons was present in every untreated animal regardless of whether their discharge was evoked by stimulation of medullary, descending spinal, or spinal reflex pathways. The failure of decerebration, spinal transection at upper cervical or lower thoracic levels, or complete bilateral lumbosacral dorsal rhizotomy to alter the degree of inhibition eliminates the possibility of its generation by supraspinal or peripheral influences. Therefore, the data indicate that sacral parasympathetic preganglionic neurons of the cat are under a strong tonic inhibition which is generated locally within the sacral spinal cord and is mediated by both GABA and glycine; disinhibition of these neurons can be accomplished only by simultaneous blockade of both inhibitory amino acids. The consistent failure of either antagonist alone to permit marked increases in evoked responses indicates that each amino acid normally produces nearly maximal inhibition of the sacral parasympathetic neurons.

The ability to increase markedly the size of evoked parasympathetic discharges by convulsant drugs greatly facilitated characterization of the pathways that were stimulated and of the neural responses that were recorded. Although the convulsants did not alter the latencies of evoked responses, they permitted the latencies to become more distinct. The long latency of the spinal reflexes indicates that their central pathways are probably multisynaptic. Estimates for the conduction velocity of the excitatory bulbospinal pathway, determined by either medullary or intraspinal stimulation, were in good agreement at 4.5-5.5 m/sec. Whether the pathway innervates the sacral autonomic neurons directly or through interneurons remains uncertain. An interesting feature of the evoked pelvic nerve discharges was their long duration which was evident in untreated animals and was prolonged by the convulsants. The duration of these responses contrasts with the shorter duration of similarly evoked responses from either motoneurons or sympathetic preganglionic neurons and suggests a different type of synaptic organization. Parasympathetic neurons may tend to discharge repetitively in response to a single volley or they may be subject to reverberating circuitry. Regardless of the underlying mechanism, the sacral parasympathetic outflow appears to be organized for comparatively slow, sustained discharge as befits its physiological role in controlling visceral functions such as bladder contraction or rectal mobility.

Many of the conclusions from the present experiments are supported by results obtained with a different approach by other investigators. de Groat (15) studied the effects of iontophoretic application of glycine, GABA, strychnine and picrotoxin to sacral parasympathetic preganglionic neurons. Whereas both glycine and GABA depressed spontaneous and D, L-homocysteic acid-evoked firing of parasympathetic neurons, strychnine reversibly antagonized only the effects produced by glycine. Although those results agree with those obtained in the present investigation, de Groat's findings that picrotoxin failed to modify responses to either inhibitory amino acid do not. In another study, Ryall and de Groat (42) investigated the effects of iontophoretic application of NE and 5-HT on sacral parasympathetic preganglionic neurons. Their conclusion that NE and 5-HT usually depressed these neurons is consistent with the present results.

The basic physiology of micturition is generally outlined as follows: various brain centers monitor the degree of bladder filling through the activity of stretch receptors located in the vesical wall. At a certain bladder pressure and volume, impulses generated by the stretch receptors are integrated by higher brain centers and eventually activate the medullary vesico-constrictor centers. Under appropriate circumstances, impulses from the vesico-constrictor centers activate the parasympathetic preganglionic neurons in the sacral intermediolateral columns and, in turn, induce contraction of the bladder.

Although higher brain centers have an important role in regulating micturition, experimental evidence indicates that they are not necessarily required. Barrington (1) observed decerebrate cats for as long as 40 hours, and although there appeared to be a slight increase in the frequency of micturition, the act of micturition was performed normally. After spinal transection, however, micturition becomes very difficult to evoke. In the present investigations, train stimulation of the descending vesico-constrictor pathways in spinal preparations never produced micturition in the untreated cat. Friedman and coworkers (21) attempted to evoke micturition in spinal cats by stimulating with electrodes applied either to the surface or inserted into the sacral spinal cord. Voiding was produced only by stimulation in the sacral intermediolateral columns as verified histologically. Although activation of pathways to the sacral autonomic neurons was ineffective in these animals, direct stimulation of the parasympathetic preganglionic neurons and their axons did evoke bladder contractions.

On the basis of evidence reported by other investigators and the data obtained in the present studies, the existence of a spinal mechanism that produces a strong, local tonic inhibition of sacral parasympathetic preganglionic neurons and that functions to inhibit micturition is indicated. According to the present results, this mechanism appears to involve both GABA and glycine as inhibitory transmitters. The GABA- and glycine-mediated inhibition of sacral autonomic neurons may be

produced by a self-sustaining inhibitory generator in the sacral cord (Fig. 16). Such an apparatus would explain the inability to produce disinhibition of sacral autonomic neurons by interruption of peripheral or descending pathways. Also, the presence of a brain stem system that interferes with the inhibitory generator is postulated from the evidence that decerebrate cats micturate normally. That this system is distinct from the vesico-constrictor centers is suggested by the finding that repetitive stimulation of either the vesico-constrictor centers or their descending pathways could not disinhibit the sacral autonomic neurons.

Decentralization of the postulated inhibitory generator may alter its influence on sacral autonomic neurons to an extent that has important clinical significance. Following spinal transection above the lower lumbar level in animals or humans, an inability to micturate persists for some weeks (19,25,41). If the bladder and kidneys are maintained in a healthy state by intermittent emptying via a catheter, spinal reflex inputs eventually become capable of producing good functional micturition. Often a state of recovery is attained whereby almost complete evacuation of the bladder can be accomplished reflexly by stimulation of perineal, perianal or other cutaneous receptors. In paraplegics, the emergence of this reflex often determines their life expectancy as urinary retention and chronic catheterization invariably lead to infection and pyelonephritis (43). It can be speculated that in cases of cord

transection above the sacral autonomic neurons, decentralization of the inhibitory generator eventually leads to its diminition or possible extinction. As this occurs, the normally weak peripheral excitatory inputs to the sacral autonomic neurons, previously ineffective inthe presence of the inhibitory generator, gradually become effective in activating these neurons and producing a functional micturition by spinal reflex pathways (Fig. 16).

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Fig. 1. Schematic representation of pathways utilized to evoke sacral parasympathetic preganglionic discharges recorded from the left pelvic nerve (S_2). Bipolar, tungsten microelectrodes were used to stimulate the medullary vesico-constrictor center or its descending pathway (T_8) which partially decussates in the lumbar cord. Spinal reflexes were evoked by stimulation of sacral afferent fibers (S_2) with bipolar, silver wire electrodes. The contralateral pelvic nerve remained intact. In some experiments increases in bladder pressures evoked by stimulus trains were measured visually from a vertical, graduated tube attached to a urethral cannula.

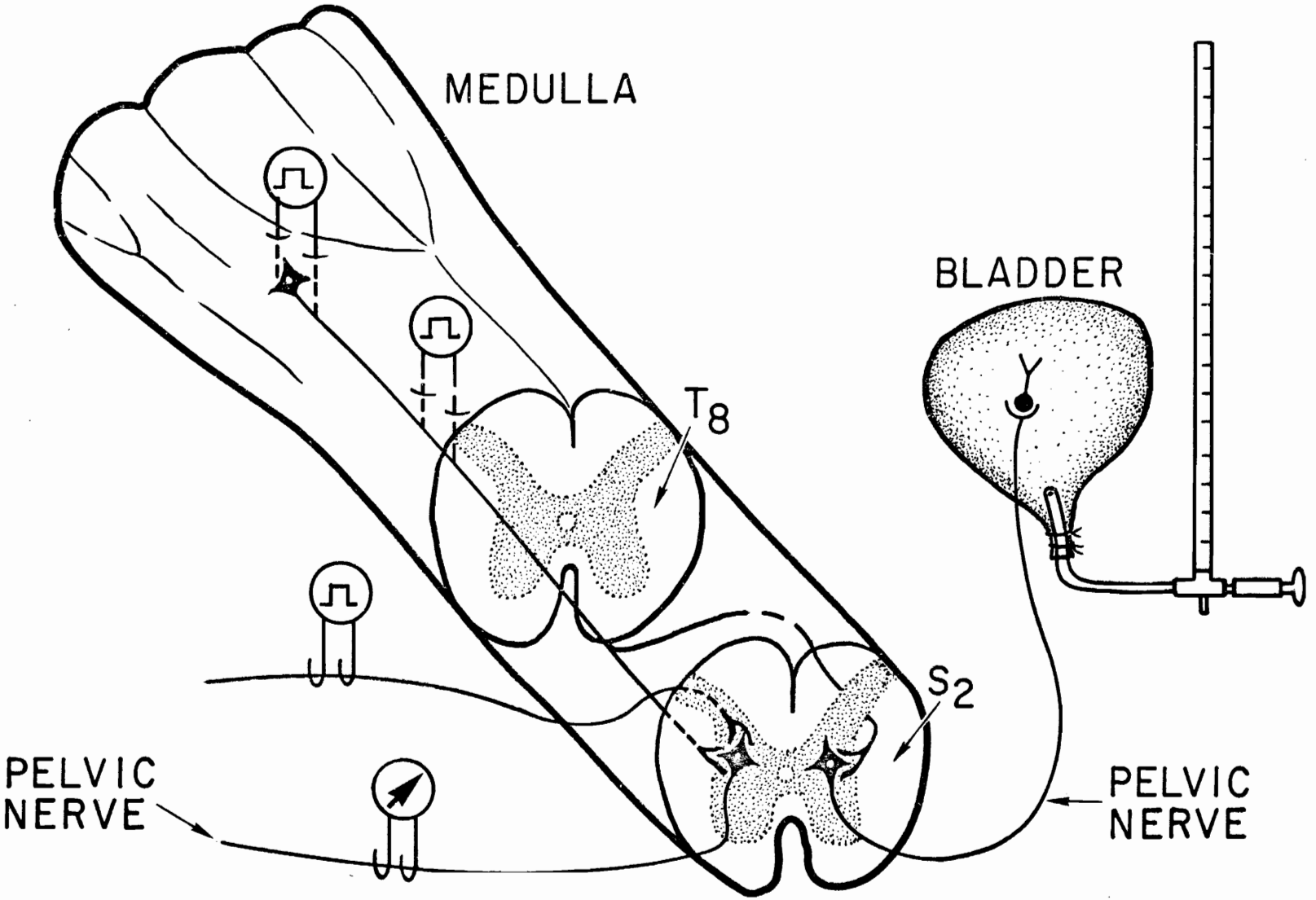

MEDULLA
BLADDER
T8
S2
PELVIC NERVE
PELVIC NERVE

Fig. 2. Representative pelvic nerve responses evoked from various pathways in untreated animals. Upper traces are individual responses and lower traces are computer averages of 32 consecutive responses. A. Spinal reflex response evoked by stimulation of sacral afferent fibers. B. Response evoked by microelectrode stimulation of the dorsolateral spinal cord at T_8. C. Response evoked by microelectrode stimulation of the vesico-constrictor center in the lateral reticular formation of the medulla. Vertical calibration represents 20 μV for upper trace of each pair. Time marker is 40 msec in A and 100 msec in B and C.

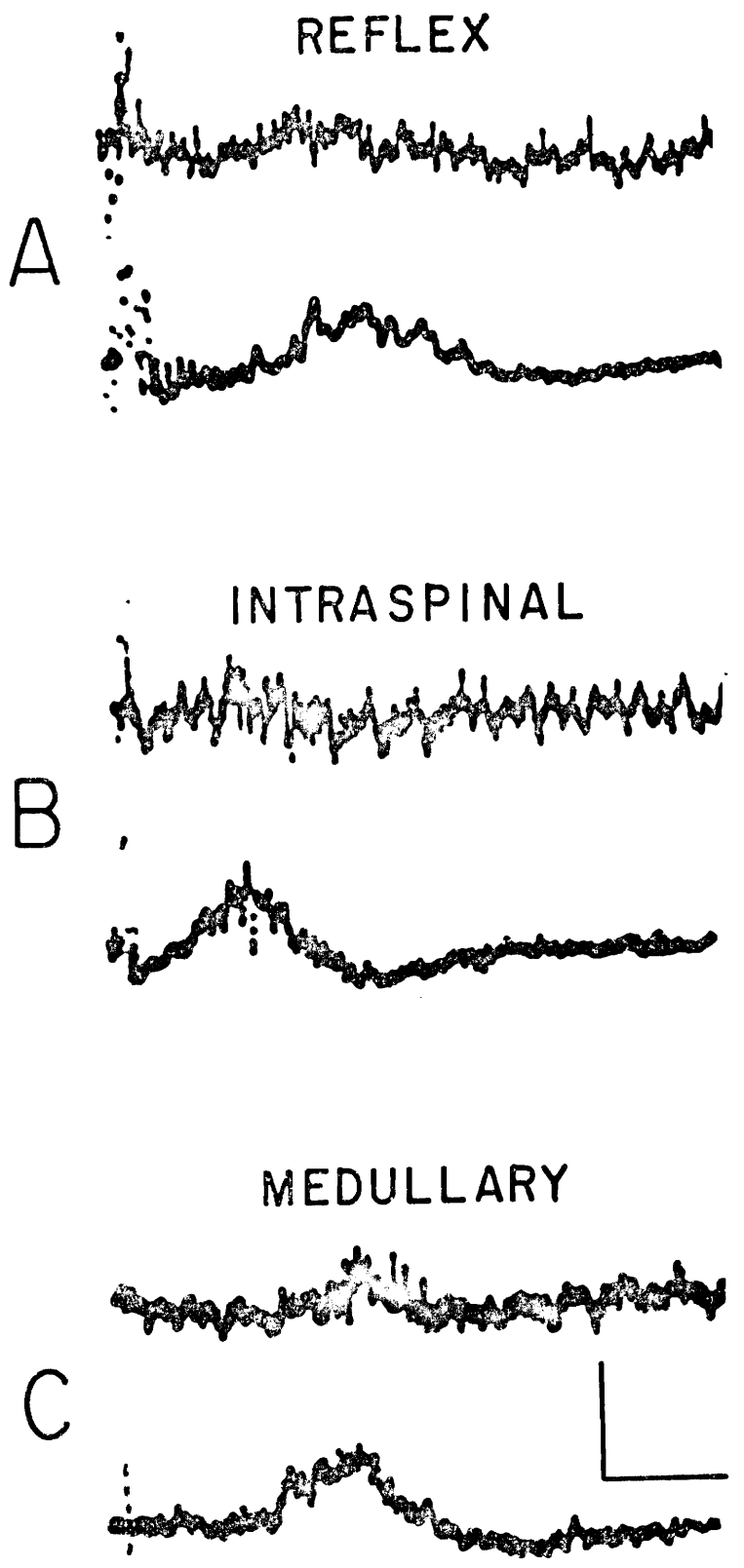

REFLEX
A
INTRASPINAL
B
MEDULLARY
C

Fig. 3. Effect of picrotoxin and strychnine on evoked pelvic nerve discharges. The upper trace of each pair is a single response, and the lower trace is a computer sum of 32 consecutive responses. A illustrates the pelvic nerve response to stimulation of the medullary vesicoconstrictor center before (control), after picrotoxin, and after subsequent administration of strychnine. Picrotoxin (2 mg/kg) was injected twice, at 0 time and at 10 min., and the recording was obtained at 12 min. Strychnine (0.2 mg/kg) was injected at 20 min. and the recording was obtained at 25 min. B illustrates the pelvic nerve response evoked by stimulation of the descending spinal pathway at T_8 before (control), after strychnine, and after subsequent administration of picrotoxin. Strychnine (0.2 mg/kg) was injected at 0 time, and the recording was obtained at 12 min. Picrotoxin (2 mg/kg) was injected at 15 min., and the recording was obtained at 23 min. Vertical calibration represents 20 μV in upper traces. Horizontal calibration represents 40 msec in A and 100 msec in B.

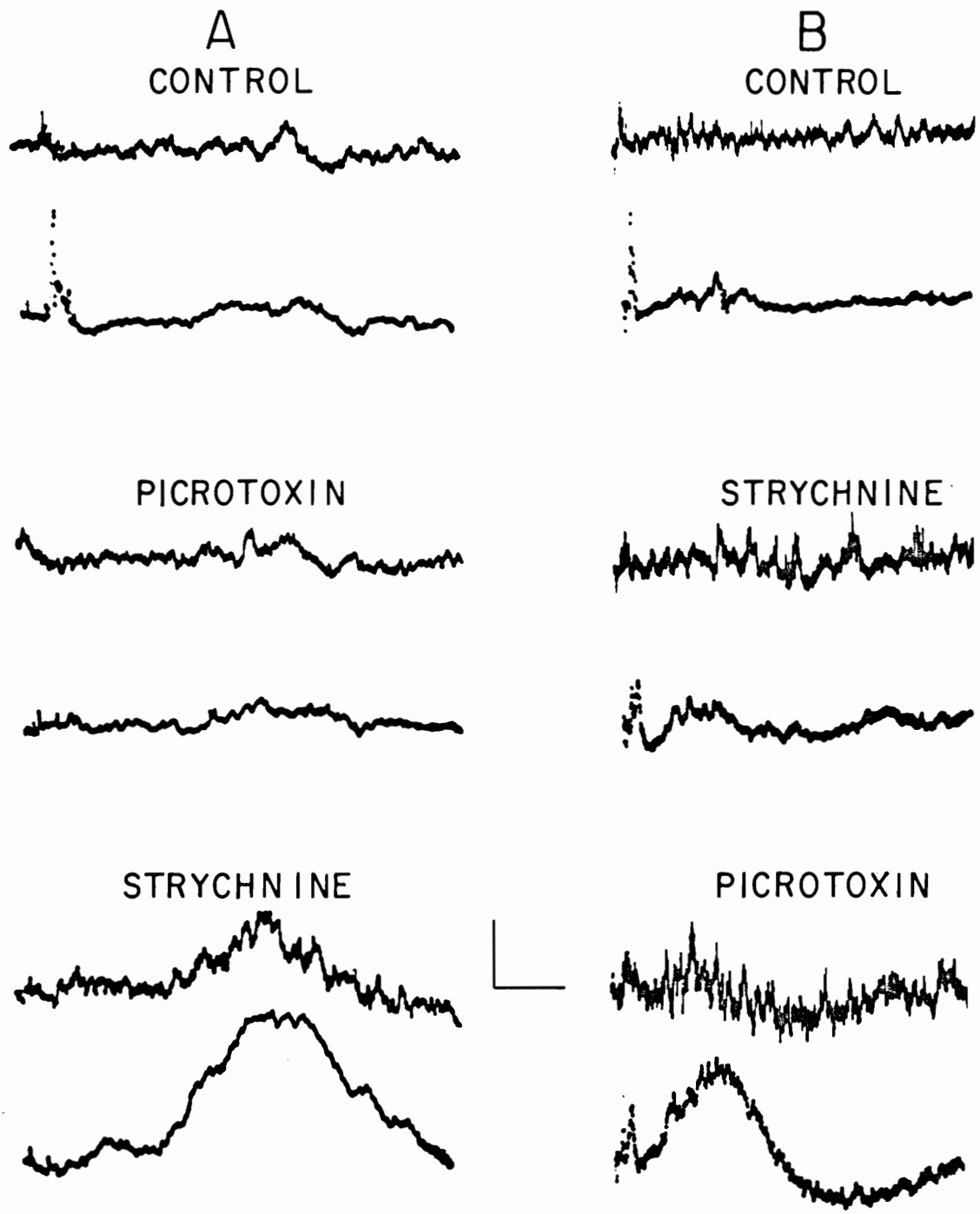

A
CONTROL
B
CONTROL
PICROTOXIN
STRYCHNINE
STRYCHNINE
PICROTOXIN

Fig. 4. Effects of picrotoxin and subsequent strychnine administration on the size of pelvic nerve responses evoked by stimulation of descending spinal (T_8) pathways. All values were obtained by integrating computer sums of 32 consecutive responses and were converted to percentages of the mean control value determined from repeated control measurements for one hour prior to 0 time.

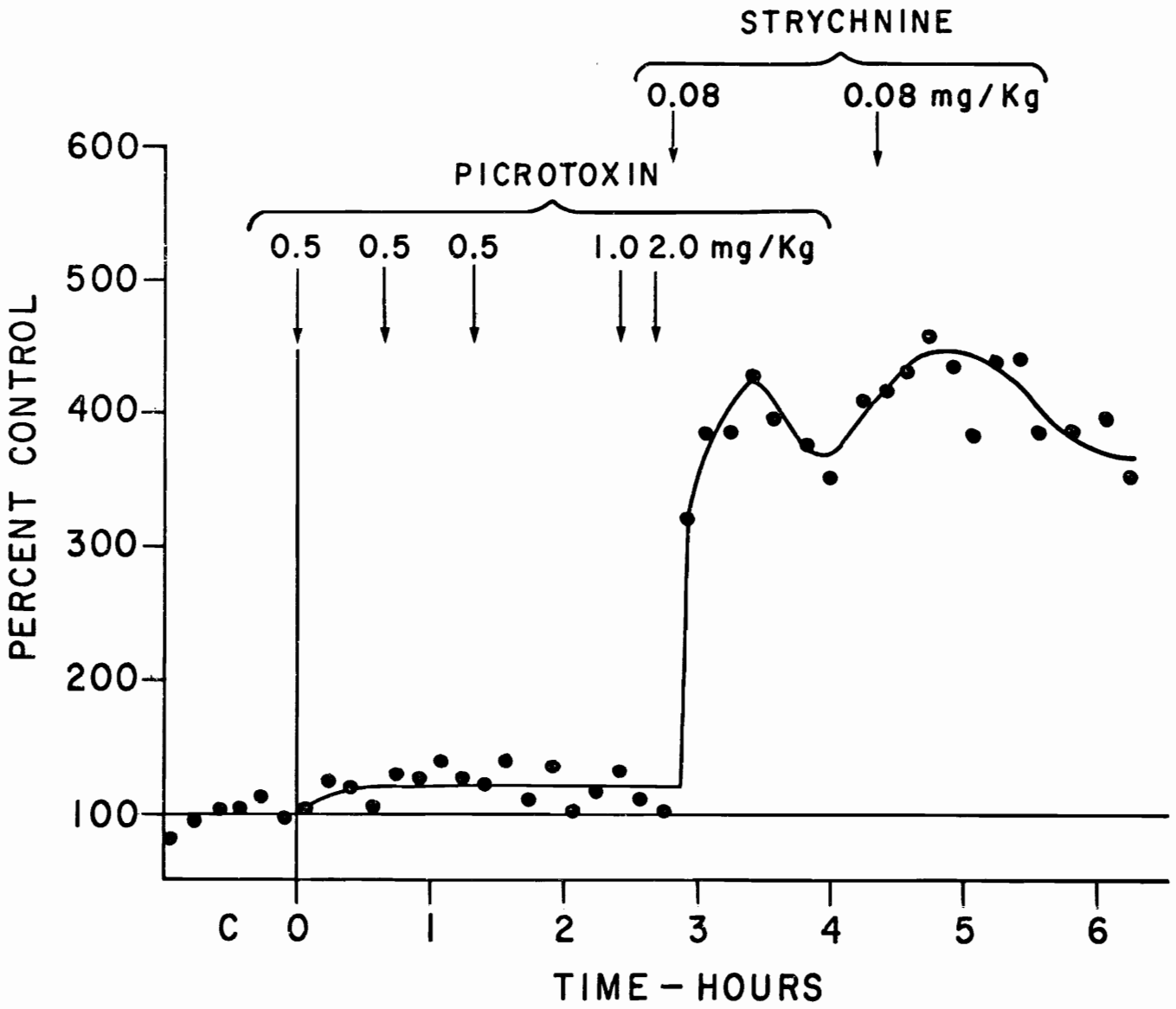

STRYCHNINE
0.08
0.08 mg/Kg
PICROTOXIN
0.5
0.5
0.5
1.0
2.0 mg/Kg
600
500
400
300
200
100
PERCENT CONTROL
C
0
1
2
3
4
5
6
TIME – HOURS

Fig. 5. Effects of strychnine and subsequent picrotoxin administration on pelvic nerve activity evoked by stimulation of descending spinal (T_8) pathways. Plotted as in Fig. 4.

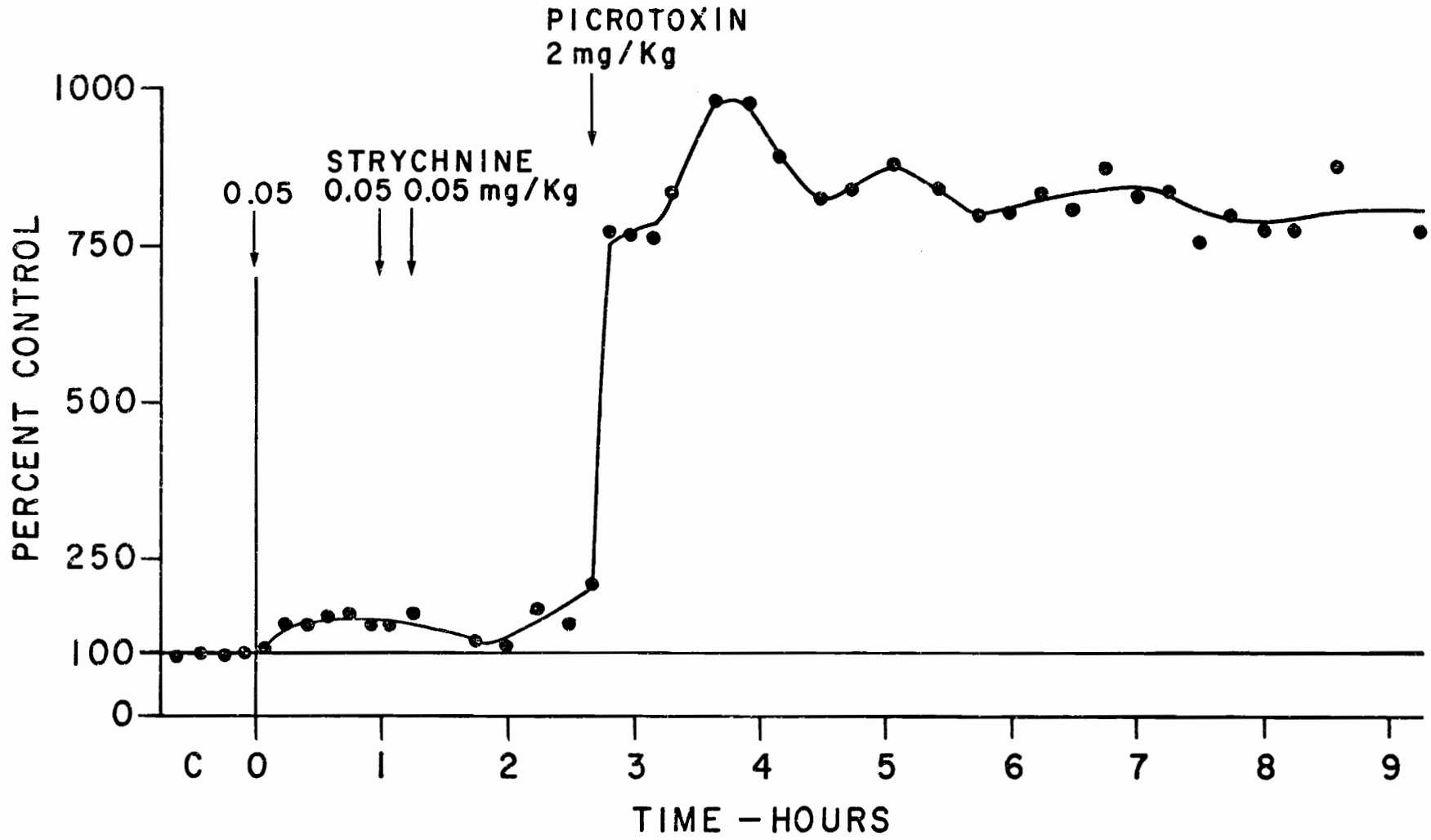

PICROTOXIN
2 mg/Kg
STRYCHNINE
0.05 0.05 mg/Kg
0.05
1000
750
500
250
100
0
PERCENT CONTROL
C 0 1 2 3 4 5 6 7 8 9
TIME – HOURS

Fig. 6. Effects of bicuculline and semicarbazide in combination with strychnine on pelvic nerve responses evoked by stimulation of descending spinal (T_8) pathways. The upper trace of each pair is a single response, and the lower trace is a computer sum of 32 consecutive responses. A shows the effect of strychnine and subsequent administration of bicuculline on the response. Strychnine (0.1 mg/kg) was injected twice, at 0 time and at 15 min., and the recording was obtained at 18 min. Bicuculline (0.5 mg/kg) was injected at 20 min., and the recording was obtained at 55 min. A large, prolonged increase in spontaneous activity after bicuculline precluded earlier collection of reliable data in this experiment. B shows the effect of semicarbazide on the subsequent response to strychnine. Semicarbazide (200 mg/kg) was injected at 0 time, and the recording was obtained at 3.5 hr. Strychnine (0.1 mg/kg) was injected at 4 hr., and the recording was obtained at 4.5 hr. Vertical calibration represents 20 μV for upper traces. Horizontal calibration represents 40 msec for all traces.

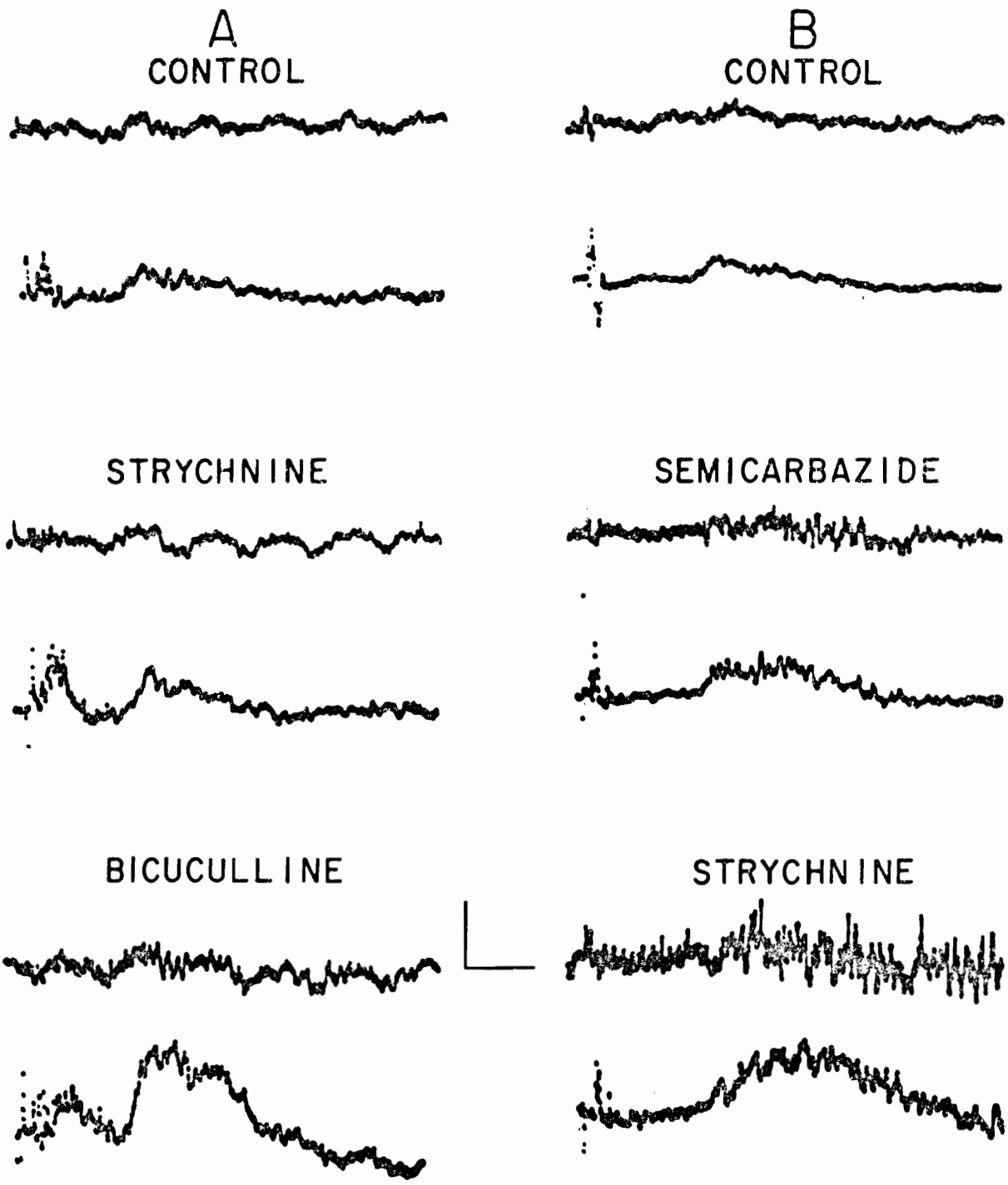

A
CONTROL
B
CONTROL
STRYCHNINE
SEMICARBAZIDE
BICUCULLINE
STRYCHNINE

Fig. 7. Effects of bicuculline and strychnine on pelvic nerve activity evoked by stimulation of descending spinal (T_8) pathways. A. Effect of strychnine followed by bicuculline administration. B. Effect of bicuculline and subsequent strychnine administration. Plotted as in Fig. 4.

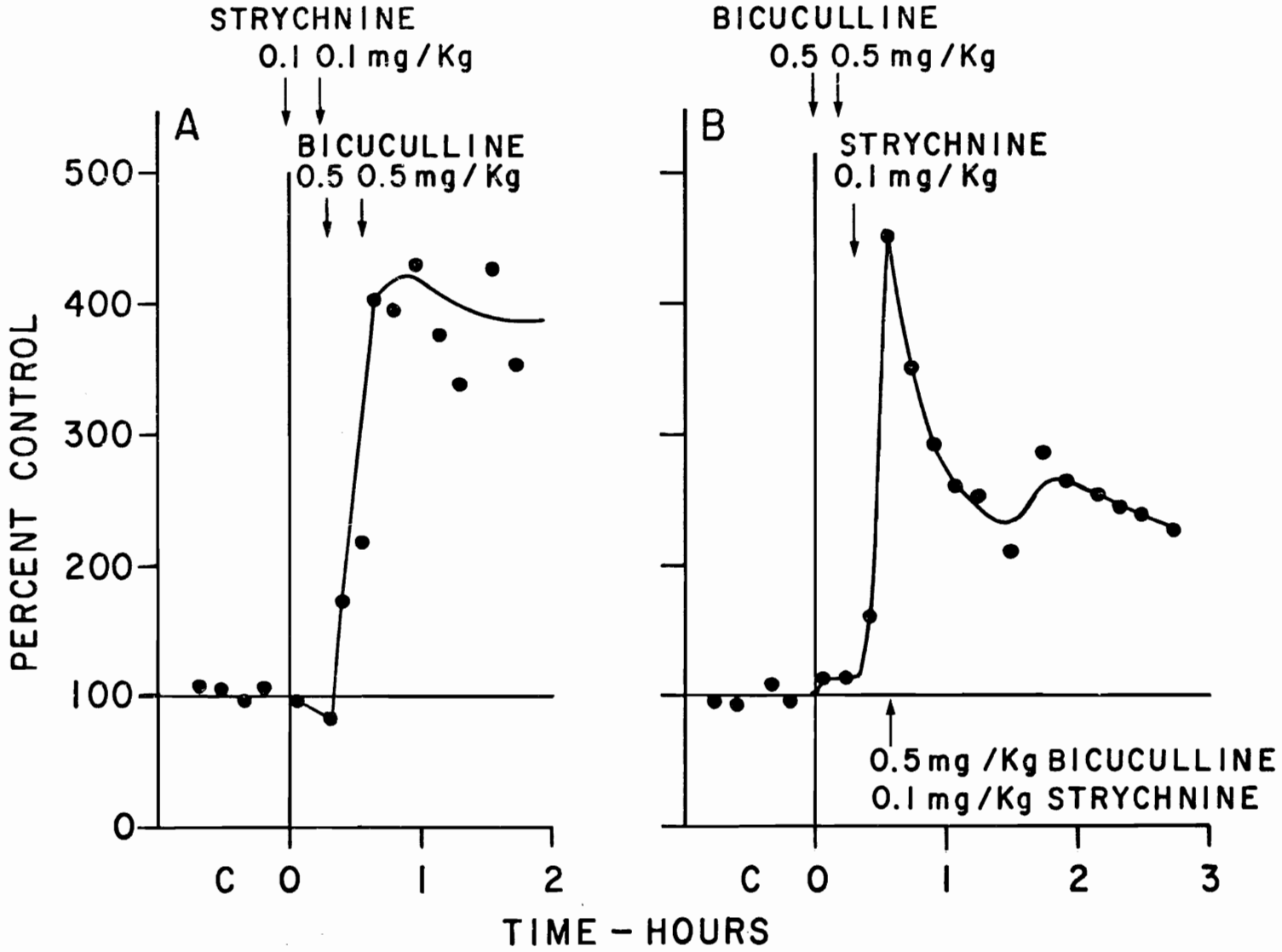

STRYCHNINE
0.1 0.1 mg/Kg
A
BICUCULLINE
0.5 0.5 mg/Kg
500
400
300
200
100
0
PERCENT CONTROL
C 0 1 2
BICUCULLINE
0.5 0.5 mg/Kg
B
STRYCHNINE
0.1 mg/Kg
0.5 mg/Kg BICUCULLINE
0.1 mg/Kg STRYCHNINE
C 0 1 2 3
TIME - HOURS

Fig. 8. Effect of strychnine on pelvic nerve activity evoked by stimulation of descending spinal (T_8) pathways following depletion of GABA stores by semicarbazide.

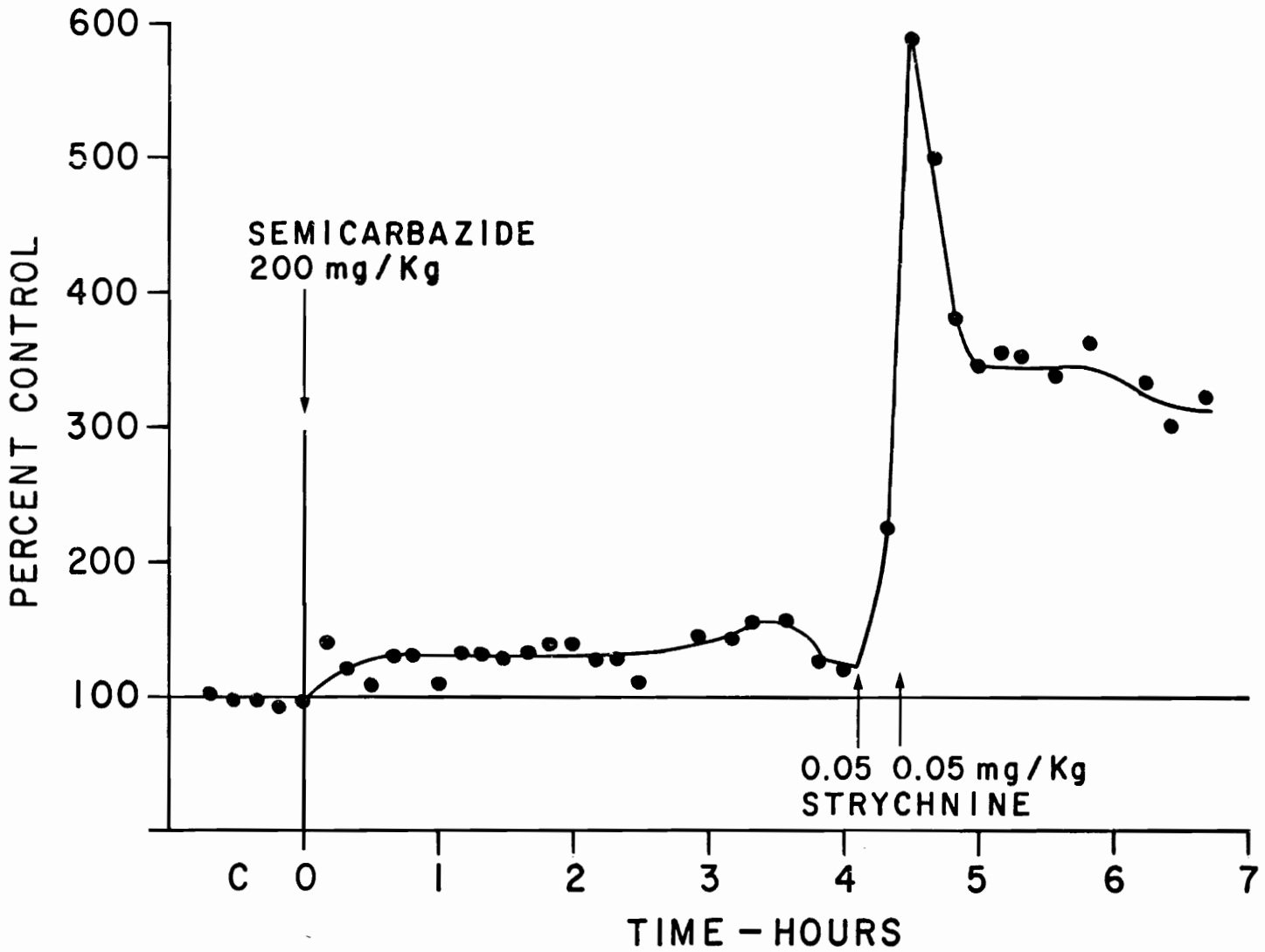

600
500
400
300
200
100
PERCENT CONTROL
SEMICARBAZIDE
200 mg/Kg
0.05 0.05 mg/Kg
STRYCHNINE
C 0 1 2 3 4 5 6 7
TIME - HOURS

Fig. 9. Effect of 5-HTP on pelvic nerve discharges evoked by stimulation of descending spinal (T_8) pathways. A. Typical time course of depression following 5-HTP (50 mg/kg). B. Effect of 5-HTP (5 mg/kg) following monoamine oxidase inhibition with pargyline. This dose is ineffective alone. Dashed lines indicate 95% confidence limits of the mean control value.

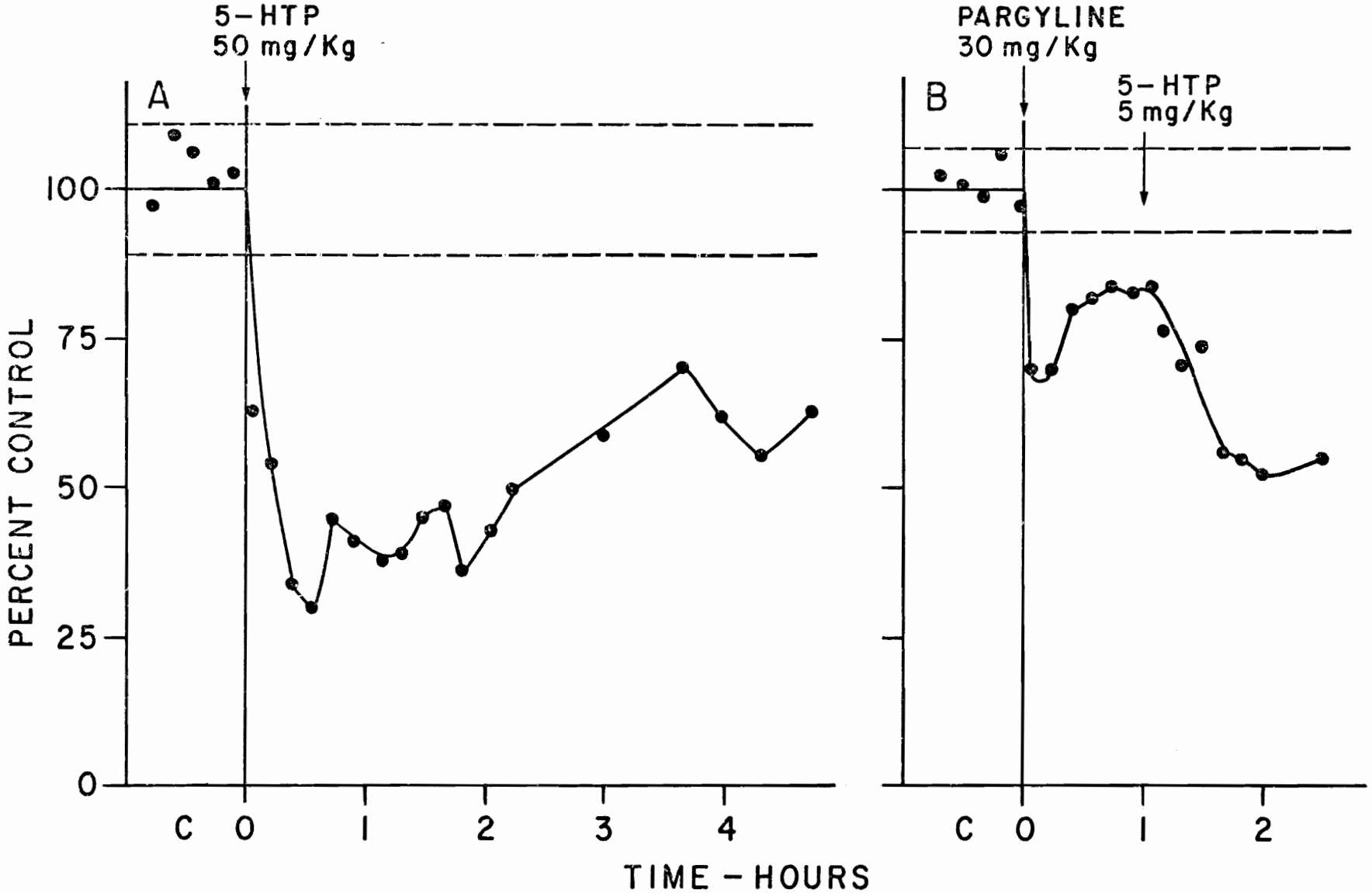

5-HTP
50 mg/Kg
A
100
75
50
25
0
PERCENT CONTROL
C
0
1
2
3
4
TIME - HOURS
PARGYLINE
30 mg/Kg
B
5-HTP
5 mg/Kg
C
0
1
2

Fig. 10. A illustrates the depression of spinal parasympathetic reflexes after 5-HTP during their enhancement by a combination of strychnine and picrotoxin. The depression was augmented by chlorimipramine. B shows the depression of spinal parasympathetic reflexes produced by chlorimipramine (5 mg/kg) alone and its potentiation by a relatively small dose of 5-HTP (35 mg/kg). Dashed lines in B indicate 95% confidence limits of the mean control value.

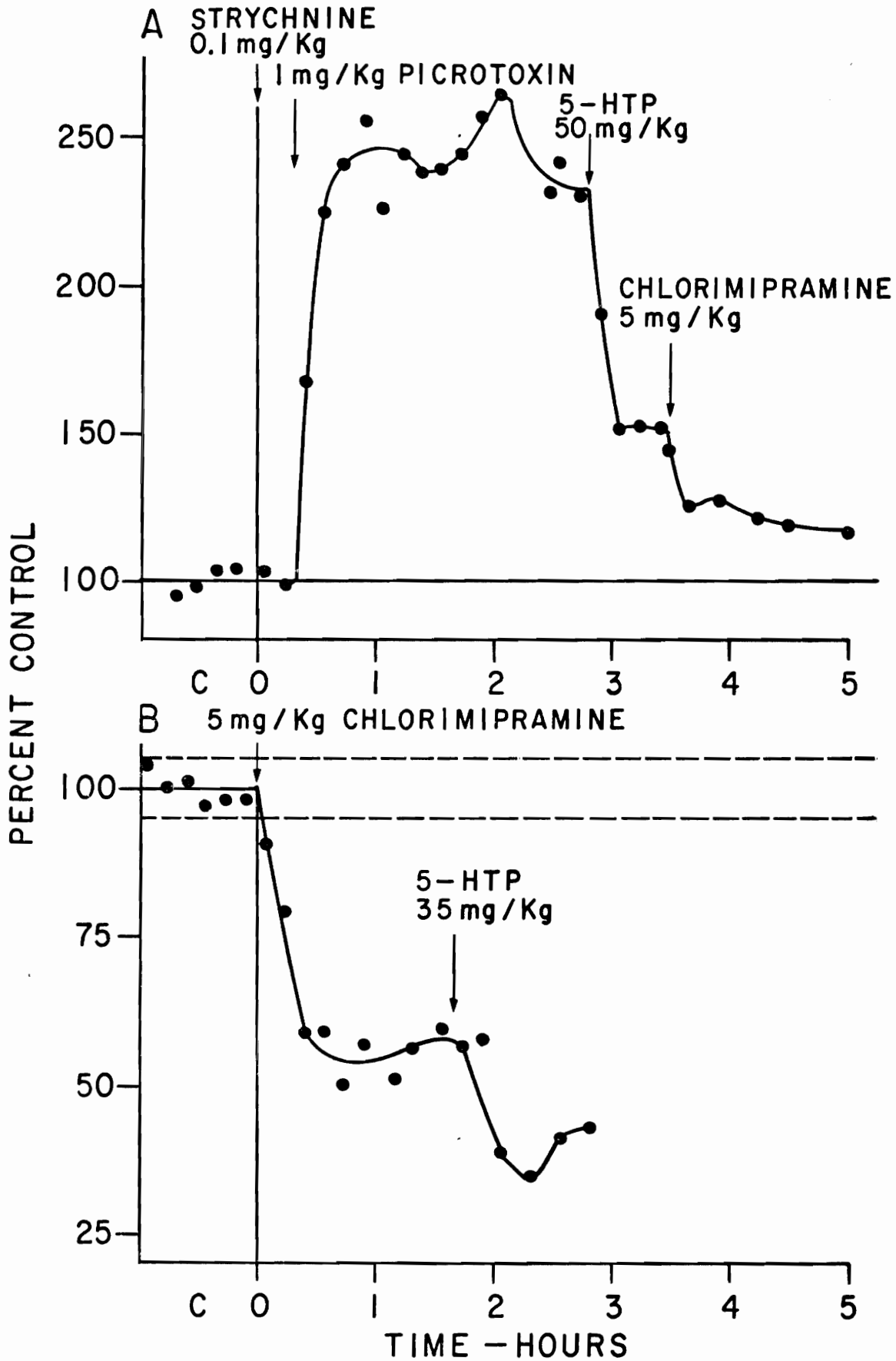

A STRYCHNINE
0.1 mg/Kg
1 mg/Kg PICROTOXIN
5-HTP
50 mg/Kg
CHLORIMIPRAMINE
5 mg/Kg
250
200
150
100
C 0 1 2 3 4 5
B 5 mg/Kg CHLORIMIPRAMINE
100
5-HTP
35 mg/Kg
75
50
25
C 0 1 2 3 4 5
PERCENT CONTROL
TIME — HOURS

Fig. 11. Depression of spinal parasympathetic reflexes by clonidine and its rapid reversal by tolazoline. Dashed lines indicate 95% confidence limits of the mean control value.

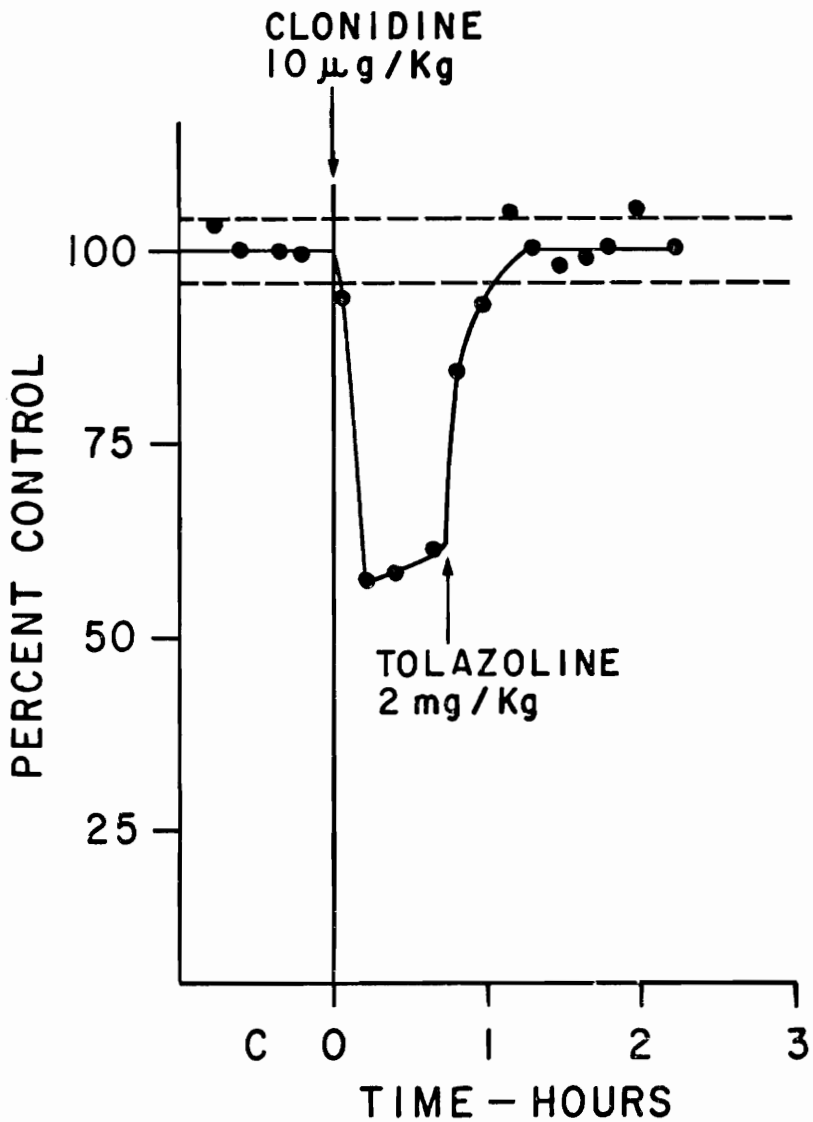

CLONIDINE
10 µg/Kg
100
75
50
25
PERCENT CONTROL
TOLAZOLINE
2 mg/Kg
C
0
1
2
3
TIME – HOURS

Fig. 12. Time course of L-dopa-induced depression of spinal parasympathetic reflexes. Dashed lines indicate 95% confidence limits of the mean control value.

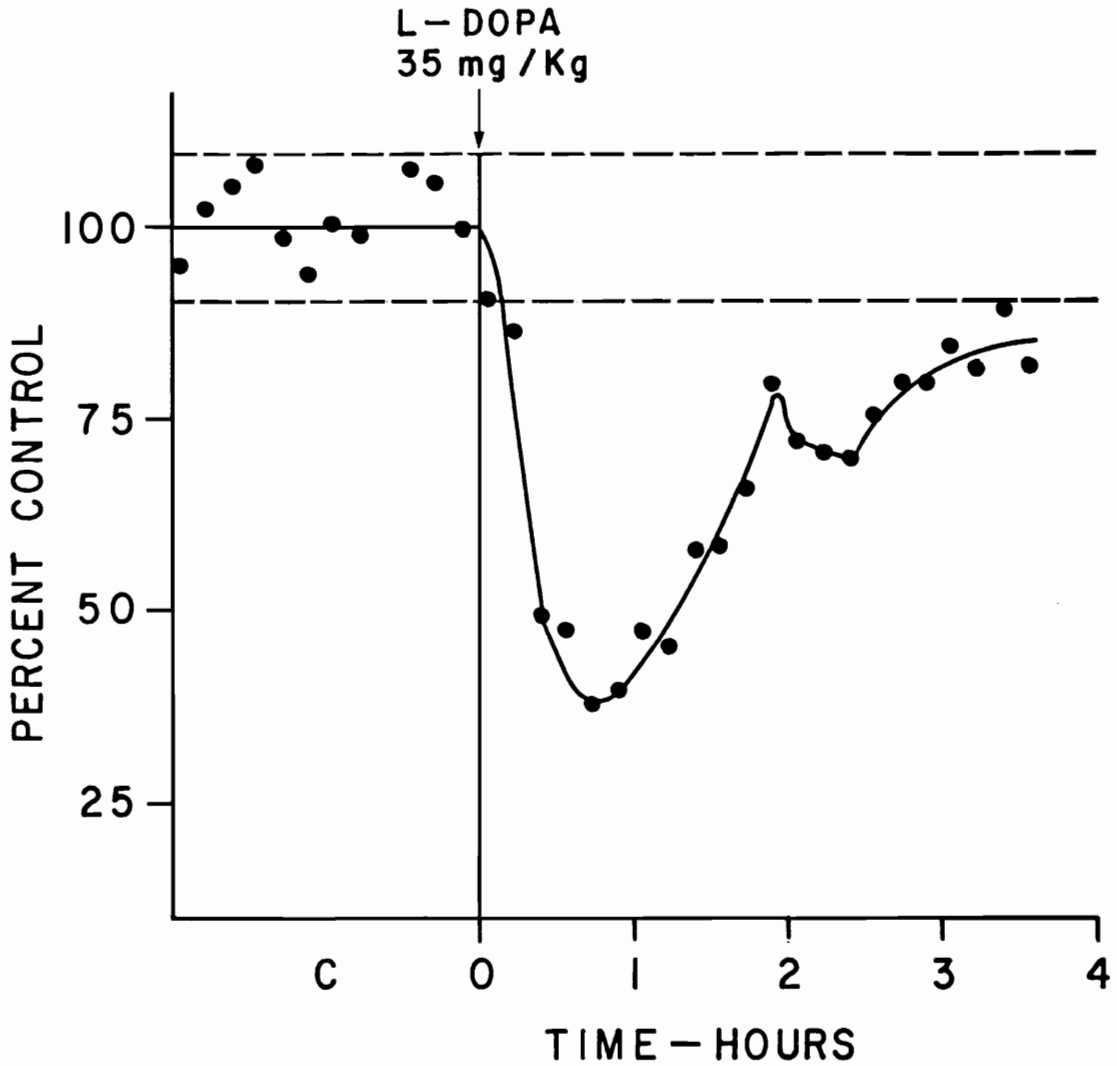

L – DOPA
35 mg / Kg
100
75
50
25
PERCENT CONTROL
C
0
1
2
3
4
TIME – HOURS

Fig. 13. Demonstration of the direct effects of L-dopa on pelvic nerve responses evoked by stimulation of descending spinal (T_8) pathways. A illustrates the failure of tolazoline pretreatment to modify L-dopa depression. B demonstrates typical L-dopa-induced depression after depletion of central 5-HT stores by parachlorophenylalanine (PCPA). Responses in both experiments were augmented by picrotoxin and strychnine.

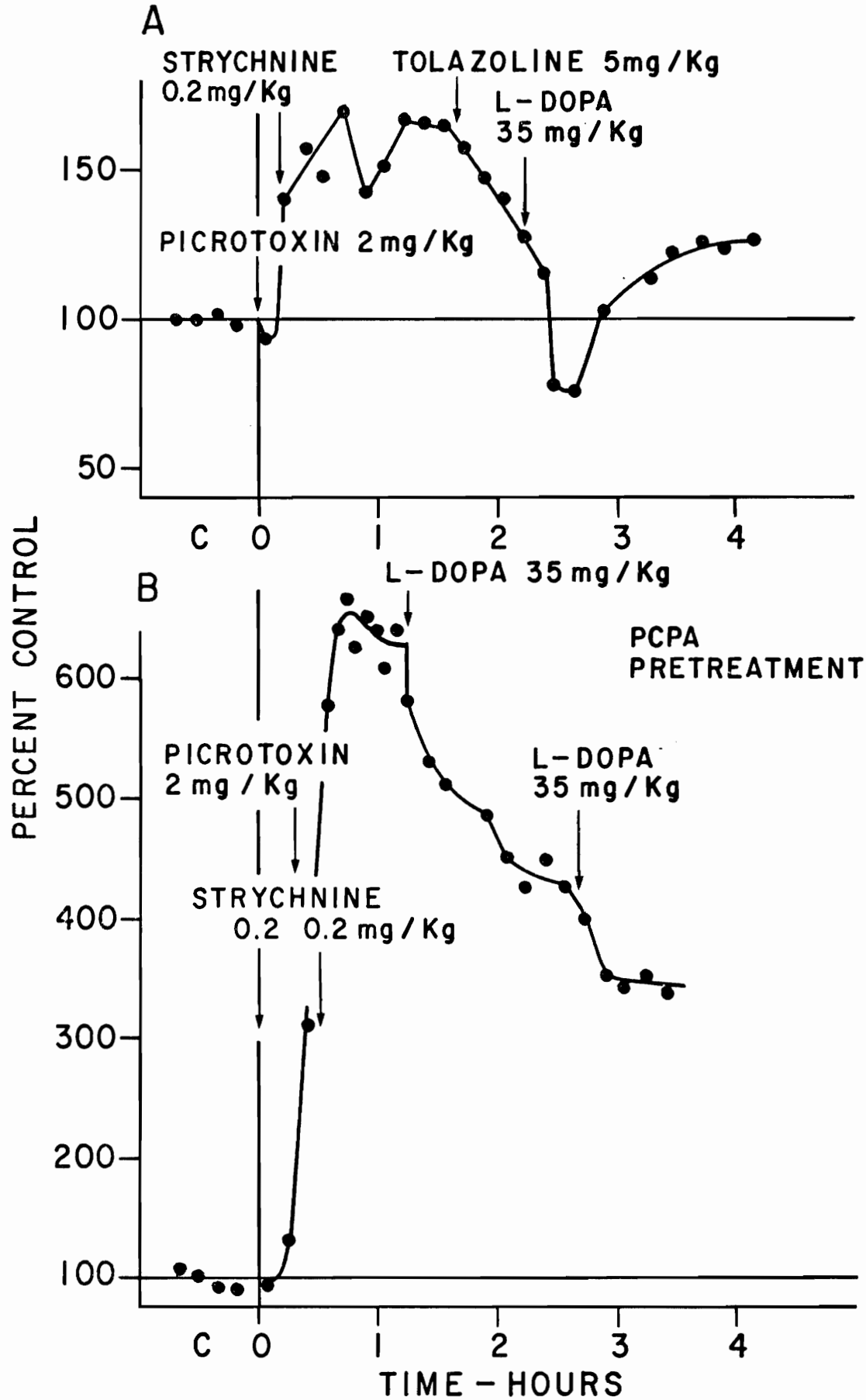

A
STRYCHNINE
0.2 mg/Kg
TOLAZOLINE 5mg/Kg
L-DOPA
35 mg/Kg
PICROTOXIN 2 mg/Kg
150
100
50
C
0
1
2
3
4
B
L-DOPA 35 mg/Kg
PCPA
PRETREATMENT
PICROTOXIN
2 mg/Kg
L-DOPA
35 mg/Kg
STRYCHNINE
0.2 0.2 mg/Kg
600
500
400
300
200
100
PERCENT CONTROL
C
0
1
2
3
4
TIME - HOURS

Fig. 14. Protriptyline potentiation of the L-dopa-induced depression of spinal parasympathetic reflexes. A. Recovery from the depressant effects of a relatively small dose of L-dopa was prevented and the depression was prolonged by protriptyline. Dashed lines indicate 95% confidence limits of the mean control value. B. Protriptyline alone depressed the evoked response and L-dopa subsequently augmented the depression.

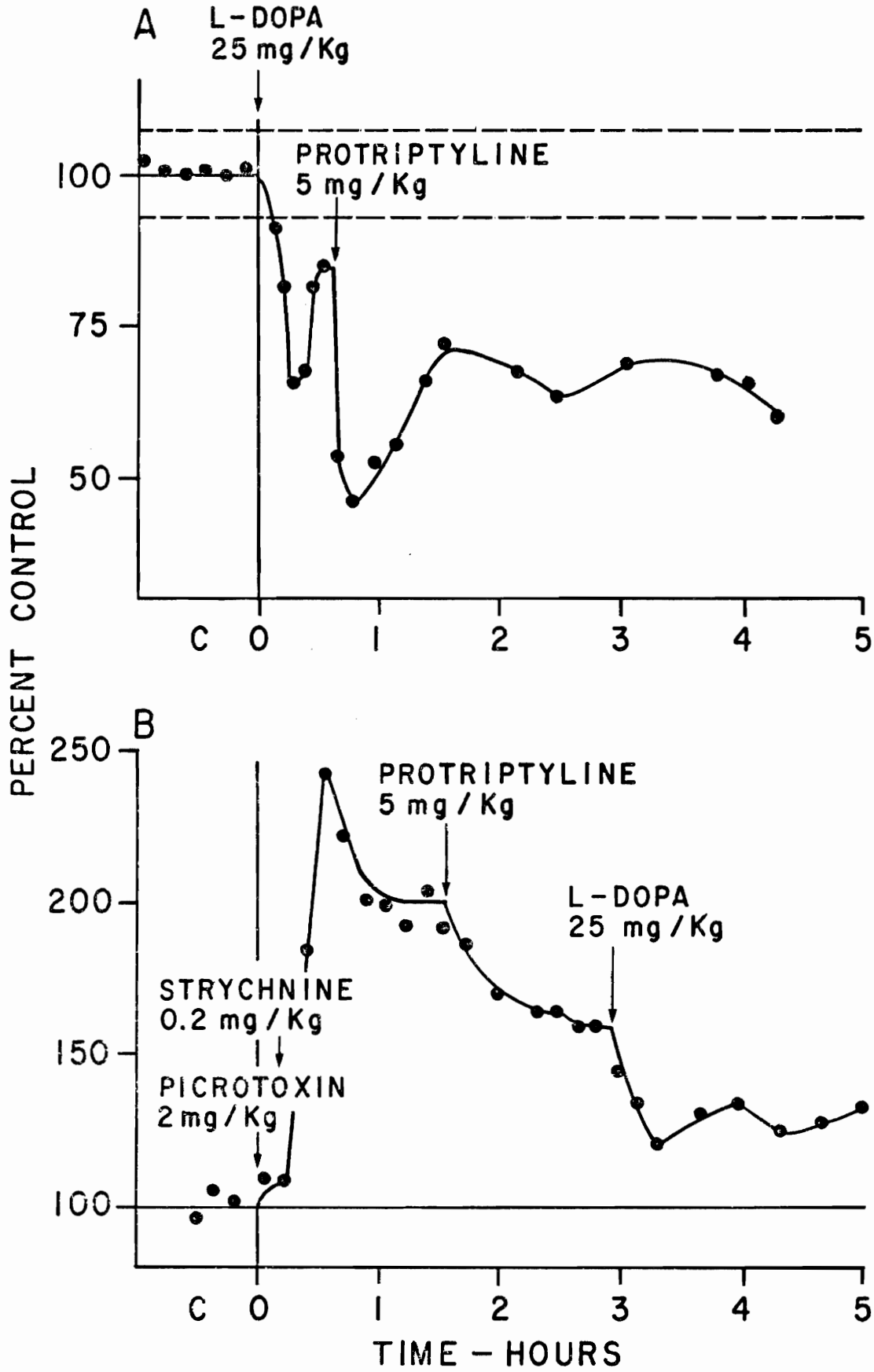

A
L-DOPA
25 mg/Kg
PROTRIPTYLINE
5 mg/Kg
100
75
50
C 0 1 2 3 4 5
B
250
200
150
100
PROTRIPTYLINE
5 mg/Kg
L-DOPA
25 mg/Kg
STRYCHNINE
0.2 mg/Kg
PICROTOXIN
2mg/Kg
C 0 1 2 3 4 5
PERCENT CONTROL
TIME - HOURS

Fig. 15. The convulsant-induced increase of pelvic nerve responses evoked by stimulation of descending spinal (T_8) pathways was rapidly depressed by reserpine. This depression, presumably due to released 5-HT and NE stores, could not be reversed with additional convulsant treatment.

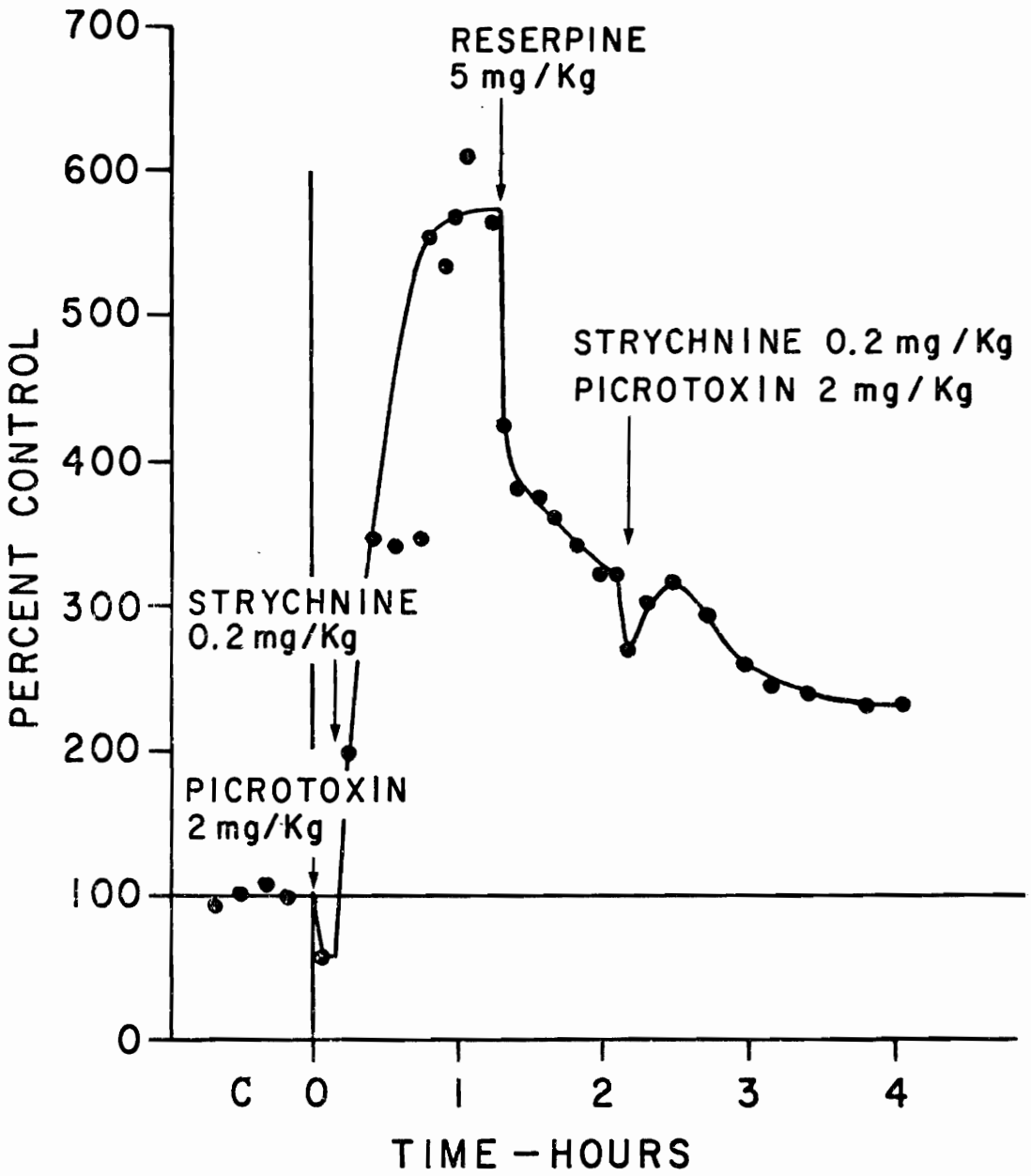
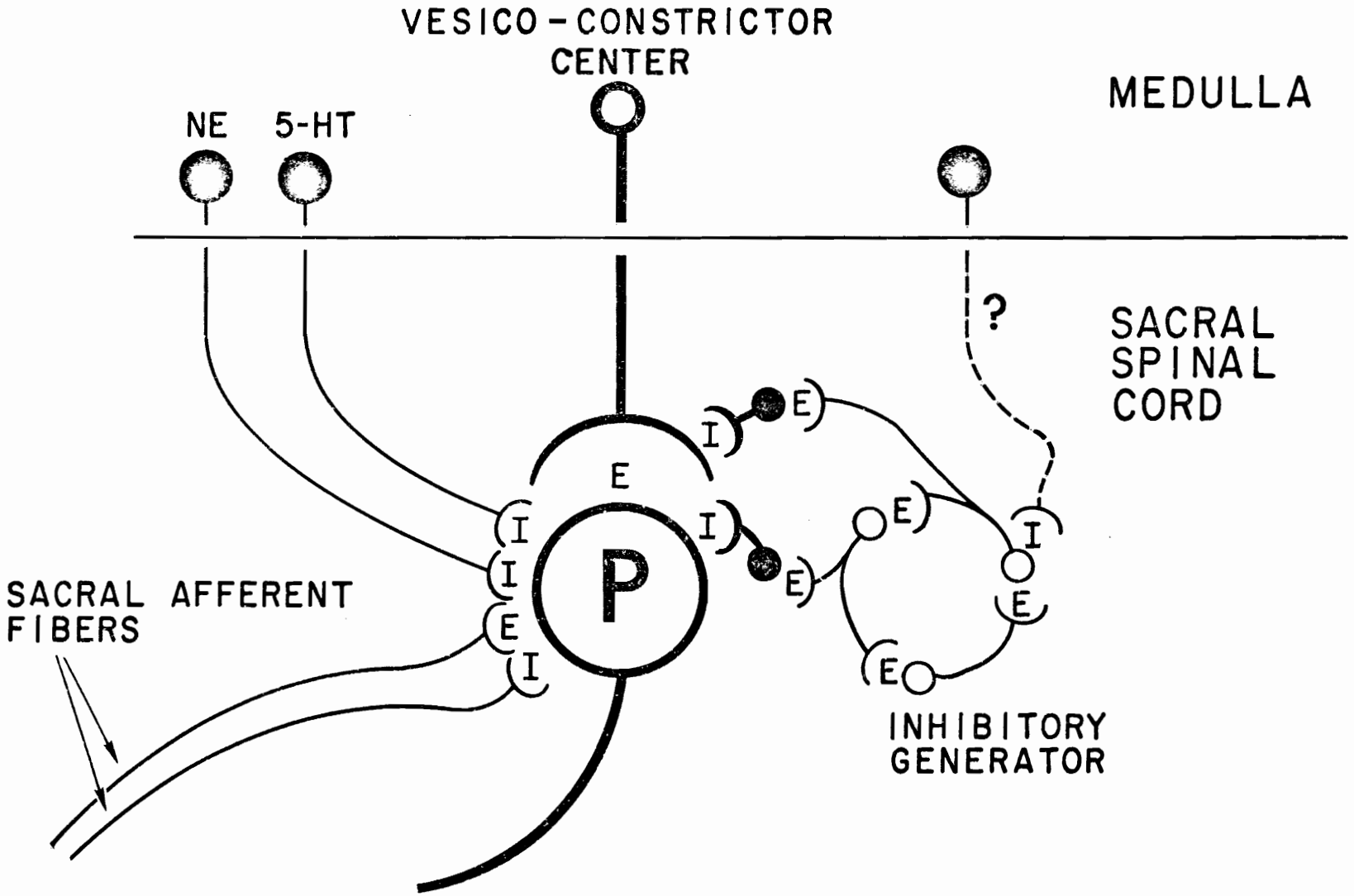

700
600
500
400
300
200
100
0
PERCENT CONTROL
RESERPINE
5 mg / Kg
STRYCHNINE 0.2 mg / Kg
PICROTOXIN 2 mg / Kg
STRYCHNINE
0.2 mg/Kg
PICROTOXIN
2 mg/Kg
C
0
1
2
3
4
TIME – HOURS

Fig. 16. A proposed schematic arrangement for the excitatory and inhibitory influences on sacral parasympathetic preganglionic neurons (P) is derived both from data obtained in the present experiments and that provided by clinical observations. Bulbosacral 5-HT and NE fibers are indicated as exerting comparatively small inhibitory influences. A proposed mechanism for the strong, local tonic inhibition of parasympathetic neurons, mediated by both GABA and glycine, is depicted by the inhibitory generator. A postulated bulbospinal control system that inhibits the inhibitory generator and thereby disinhibits the preganglionic neurons is also included.


VESICO-CONSTRICTOR
CENTER
MEDULLA
NE
5-HT
?
SACRAL
SPINAL
CORD
E
I
I
I
I
E
E
E
E
E
I
I
E
P
SACRAL AFFERENT
FIBERS
INHIBITORY
GENERATOR

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Publications

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TURKANIS, S.A., and THOMSON, T.D., Increase in transmitter release at the frog neuromuscular junction induced by the barbiturates, Fifth International Congress on Pharmacology, (1972) 238, San Francisco.

THOMSON, T.D., and FRANZ, D.N., Inhibition of Sacral Parasympathetic Preganglionic Neurons by 5-HT, NE, Glycine and GABA, submitted for ASPET Fall Meetings, (1974), Montreal.